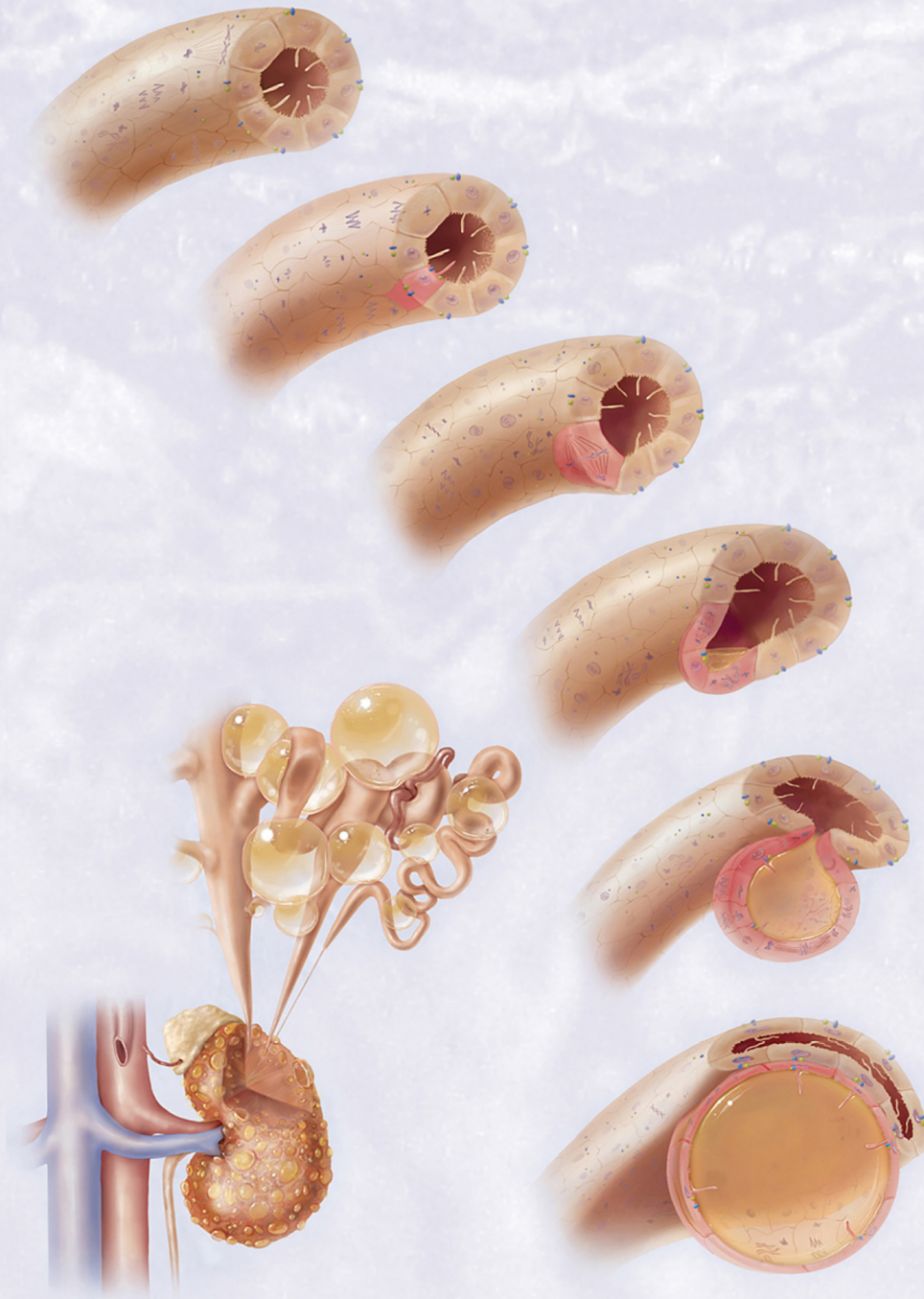


POLYCYSTIC KIDNEY DISEASE

Xiaogang Li
Editor



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Foreword

I have had the good fortune of watching, from the very beginning, the explosive increase in knowledge of renal cystic disorders over the last five decades. My personal interest began when my elementary school chum, Ronnie Wilkerson, told me he had polycystic kidney disease (PKD), knowing that one day it would take his life. Ultrasound detection of cysts had just been applied to diagnose PKD patients at the University of Colorado School of Medicine in Denver. In 1974, Joseph Holmes M.D. made the initial presentation of what has become the largest cohort of affected individuals at a single site in the United States. Ronnie's name appears at an early position in that list. In his hour of need, however, there was no research to speak of except for long essays attempting to classify all of the inherited and acquired renal cystic disorders. These ended up becoming bewildering diatribes I have referred to, in a naughty mood, as "nattering nosology".

The first successful effort to move beyond descriptive accounts of the disease can be attributed to O.Z. Dalgaard, a Danish geneticist who in 1957 defined the genetics of autosomal dominant (ADPKD), thereby proving that the etiology of the condition was harbored in defective DNA. This provided a huge lead toward finding the cause of the disease. Lacking tools to localize the mutation and determine the molecular basis of cystic disorders, a few brave souls tried to learn something about the disease pathogenesis using classic physiology and pathology investigative approaches, now considered "blue collar".

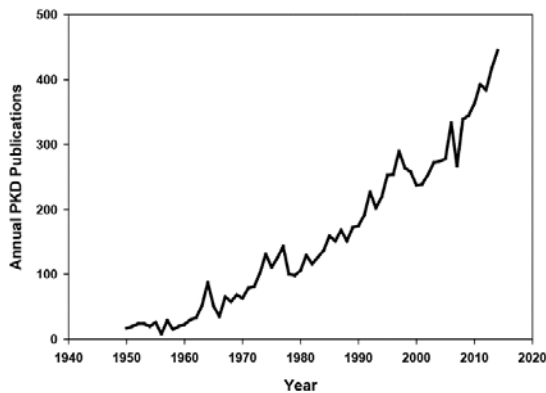
In 1969, Kenneth Gardner M.D. collected cyst fluids from a single polycystic kidney, measured the electrolytes and osmolality in them, and discovered that some of the cysts continued to function as collecting ducts in spite of their massive dilatation. Prior to that renal cysts fell under the purview of radiologists who publically referred to them as "simple", and privately as "dull and a dead issue". With Jay Bernstein M.D. and Andrew Evan Ph.D., Gardner studied animals administered chemicals that caused acquired cysts to appear and, in doing so, discovered the important role that excessive cell proliferation plays in the development of a renal cyst. Frank Carone M.D. also explored the gross and micro-anatomy of cysts, finding abnormalities in the basement membranes and the renal interstitium.

I discovered, quite by accident, that normal renal tubules could secrete fluid, a new, and not especially welcome, function for normal kidney tubules. Wondering what to do with that unexpected discovery, I remembered Ronnie Wilkerson's cystic kidneys and I drew a connection between renal fluid secretion and the likelihood that it had something to do with how the fluid gets into renal cysts. In this new light I made it a quintet of "blue collar" scientists working on PKD.

Foreword

In 1982, Joseph Breuning, a Kansas City business man, and I created the *Polycystic Kidney Research Foundation* to raise money in support of finding treatments and an eventual cure for PKD. In 1984, we invited every scientist and physician-scientist in the world who could spell polycystic to participate in a workshop to determine just how much useful information was in hand and where we needed to go to have the greatest impact. The first NIH-sponsored Program Project Grant emerged from the discussions in an atmosphere of excitement and camaraderie that motivated some exceptionally talented researchers among the attendees to join the intrepid band of PKD researchers.

From this modest beginning, PKD research has become a mainstream international field that enjoyed publication in 2012 publication of a large clinical trial showing that the blockade of vasopressin V2 receptors would slow the growth of cysts and reduce the rate of decline in glomerular filtration rate. And there certainly will be more trials and successes as detailed in this timely book, edited by Xiaogang Li. He has gathered PKD- focused scholars from all parts of the world to share their perspectives on the current diagnosis and treatment, the molecular mechanisms contributing to cyst formation and growth, and the extra-renal manifestations of PKD.



This is a volume that emphasizes new developments in the field. When one considers how much has been learned over the last four decades, compiled in the annual growth of PKD publications listed in PUB-MED (Figure), it is safe to say that it will be necessary to update this book within a few years.

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Preface

Polycystic kidney disease (PKD) is one of the most common life threatening genetic disorders caused by single-gene mutations. Its true prevalence is unknown but, based on autopsy studies, it may affect more than 100,000 persons in the United States, and millions more worldwide. It is characterized by the presence of fluid-filled cysts in the nephrons of both kidneys, eventually leading to kidney failure in the majority of affected individuals. PKD is the fourth most common cause of chronic renal insufficiency or end-stage kidney disease (ESKD). Fifty per cent of adult PKD patients will require dialysis or kidney transplantation within their 6th decade.

There are at least two major types of PKD: autosomal-dominant (AD) PKD and autosomal-recessive (AR) PKD. The genes responsible for these two types of PKD, PKD1 and PKD2 for ADPKD, and PKHD1 for ARPKD, have been identified in the past 20 years. In addition to genetic factors, molecular, cellular and epigenetic factors that contribute to the development of PKD have also been unravelled. This book focuses on the basic and clinical aspects of the burgeoning PKD research. It contains the current information on the diagnosis, management and treatment of PKD; the most recent, pertinent and comprehensive information on the mechanisms of cyst formation in PKD; and the latest information on extra-renal manifestations associated with PKD.

Section I provides a comprehensive guide to the diagnosis, management and treatment of PKD under six broad headings: differential diagnosis (chapter 1); management and treatment of childhood PKD (chapter 2); treatment and management of ADPKD (chapter 3); and diagnosis and treatment modalities for symptomatic PKD (chapter 4). Hypertension (high blood pressure) is always one of the earliest symptoms of PKD, developing in most ADPKD patients by the age of 20 or 30. Blood pressure control in PKD is discussed in chapter 5. Chapter 6 summarizes the completed, and ongoing, clinical trials in PKD, allowing basic scientists to readily view how their efforts are currently being translated to the clinic.

Section II of this book covers most of the fundamental molecular and cellular mechanisms underlying PKD and how this knowledge is contributing to the development of potential novel therapeutic agents. This will allow basic scientists and clinicians to conveniently read these side-by-side chapters to review the basis of the diseases they are studying and treating. In chapter 7, and also in chapter 2 of section 1, the authors provide a general summary of the molecular and cellular pathogenesis of childhood PKD and ADPKD, with emphasis on defective intracellular calcium homeostasis and the cellular response to cyclic AMP. These chapters also elegantly tease out the interconnected roles of MAPK/ERK (mitogen-activated protein kinase/extracellular-regulated protein kinase), mTOR (mammalian target of rapamycin), Wnt, JAK (Janus kinase)/STAT, Cdk (cyclin-dependent kinase), and EGFR (epidermal growth factor receptor) pathways in ciliary dysfunction, cyst formation, renal inflammation and fibrosis. Chapter 7 also highlights the application of atomic force microscopy and small angle X-ray scattering (SAXS) techniques

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to address polycystin multimerization properties. This chapter does not assume any previous knowledge, and gives a clear, concise summary of the key theories and the associated historical figures.

Because tolvaptan, a vasopressin V2 receptor (V2R) antagonist, is already clinically approved for the treatment of ADPKD in several countries, a chapter that comprehensively discusses the role of calcium and cyclic AMP in PKD, and the rationale for using tolvaptan to treat PKD has been included (chapter 8).

Apoptosis as a cellular mechanism in PKD has been thought to contribute to cyst expansion. It has been suggested that reduction in apoptosis was associated with a decrease in cyst growth and kidney expansion, although most of the studies were conducted in ARPKD animal models. However, recent evidence has indicated that induction of apoptosis in cyst lining epithelial cells delayed cyst growth in ADPKD animal models, suggesting that under certain conditions enhanced apoptosis may preserve renal structure by eliminating mural cells from cysts that otherwise would proliferate without limit. The roles of apoptosis in ARPKD and ADPKD animal models are extensively reviewed in chapter 9, opening this controversy to all PKD researchers and encouraging further investigation.

High expression of c-Myc has been observed in kidneys of human ADPKD patients, mouse models produced by dysregulation of *Pkd1* and *Pkd2* gene dosage, and several non-orthologous animal models of PKD. Chapter 10 summarizes the central roles of c-Myc in the pathogenesis of PKD mouse models, and in human ADPKD development and progression.

PKD1 gene product polycystin-1 can be post-translationally modified by cis-autoproteolytic cleavage at the G-protein coupled receptor proteolytic site (GPS) motif, located at the base of the extracellular ectodomain. The role of defective GPS cleavage in the pathogenesis of ADPKD is discussed in chapter 11.

Epigenetics is one of the fastest growing fields of human disease research. Chapters on epigenetics, (chapter 12), and microRNA (chapter 13) highlight how genetic make-up and epigenetics regulate gene expression and protein function. Knowledge of epigenetic factors has yielded an exciting guide to an ongoing clinical trial (chapters 6 and 12) by targeting sirtuin 1 with nicotinamide (vitamin B3) as a potential PKD therapy.

Renal inflammation has recently been linked to cyst progression in PKD. Chapter 14 highlights the roles of renal inflammation, and the involvement of the PKD1 gene in regulating the expression of some of the pro-inflammatory chemo-attractants such as monocyte chemo-attractant protein-1 (MCP-1) and cytokines such as tumor necrosis factor- α (TNF- α).

PKD has also been associated with ciliary dysfunction, and is known as a ciliopathy. Ciliopathies are genetic disorders caused by mutations that affect the structure and

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function of the cilia or basal bodies. This book has two chapters that provide a general review of the structure and mechanosensory function of primary cilium, as well as the contribution of primary ciliary dysfunction to kidney and cardiovascular disease pathogenesis associated with ADPKD (chapters 15 and 16).

Finally, section III focuses on extra-renal complications. The most common extra-renal manifestations or secondary complications associated with PKD are hypertension, acute and chronic pain, extra-renal cysts, intracranial aneurysms, kidney stones, and ESKD. Although several extra-renal manifestations of PKD are discussed in chapters 2 through 5, this section is dedicated for a special set of secondary complications: cardiovascular complications (chapter 16); PKD-associated liver cysts (chapter 17); seminal vesicle cysts in ADPKD (chapter 18); polycystin-mediated craniofacial development (chapter 19); and rapidly progressive glomerulonephritis in ADPKD (chapter 20).

Chapter 17 extensively discusses the pathogenic sequence and genetic profile of liver cyst formation and progression either as a distinct genetic disease in the absence of renal cysts or in ADPKD and ARPKD. The genetic connection between autosomal dominant polycystic liver disease (ADPLD) and ADPKD is also discussed.

Patients with ADPKD are generally known to be fertile. Women with ADPKD usually can complete successful pregnancies. However, as discussed in chapter 18, some men with ADPKD develop conditions that may affect their fertility because of necropermia (sperm in the semen that are not alive), immotile sperm, seminal vesicle cysts, and ejaculatory duct cysts.

The role of the polycystins in controlling craniofacial development and growth has recently been reported in *Pkd1* and *Pkd2* mutant mice, as discussed in chapter 19. This information may be useful in understanding the interaction between PKD and head growth and development in ADPKD patients. Many cases of rapidly progressive glomerulonephritis (RPGN) have been reported in ADPKD kidneys. As such, the discussion of RPGN in chapter 20 may help clinicians to understand the full spectrum of potential renal manifestations.

This book also provides a broad overview of some of the biggest challenges currently faced by researchers and clinicians in the PKD field. Like all good publications, the biggest problem is that it leaves you wanting more. The intended audience of this book is students, basic scientists and clinicians who are interested in the basic and/or clinical aspects of PKD. The goal of this book is that it would act as an authoritative source for readers who want a comprehensive understanding of the development, progression, management and treatment of PKD.

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Chapter 1

Differential Diagnosis of Autosomal Dominant Polycystic Kidney Disease

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the most common life-threatening hereditary disorder characterized by cyst formation and enlargement in the kidney and other organs. There are two known mutations in ADPKD: PKD1 (85% of cases), whose clinical manifestations are the earliest and most rapidly evolving; and PKD2 (15% of cases). PKD1 is a large and complex gene encoding polycystin-1, whereas PKD2 is smaller and encodes polycystin-2. There are a few patients reported in the literature who will not

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fit into any of these subgroups, leading clinicians to question the exact diagnosis, for example, patients without either of these mutations or patients with predominant development of hepatic cysts. The differential diagnosis between ADPKD and other cystic kidney diseases depends on the age of the patient, family history and the presence of associated manifestations. In adult patients in the absence of a family history of ADPKD, doctors should exclude: multiple benign simple cysts; localised or acquired renal cystic disease; medullary sponge kidney; bilateral parapelvic cysts; autosomal recessive polycystic kidney disease (ARPKD); tuberous sclerosis complex (TSC); von Hippel-Lindau disease; autosomal dominant medullary cystic disease; autosomal dominant polycystic liver disease; and X-linked dominant orofaciodigital syndrome type I. In young children, in the absence of family history of ADPKD, it is important to distinguish from ARPKD, contiguous PKD1-TSC2 syndrome or Meckel-Gruber syndrome. This chapter will review the challenges in the diagnosis of multiple kidney cysts in adults, pointing out the most important signs which doctors should be aware of to reach an appropriate diagnosis in this condition.

Key words: Differential diagnosis; PKD1; PKD2; Renal cysts

Introduction

The detection of a single or multiple kidney cysts is very common, especially with advancing age, and it has no particular significance. On the other hand, there are patients who have multiple cysts which, depending on a set of characteristics including age, the number of cysts, and their distribution across several organs, may be included in the so-called polycystic kidney diseases. These diseases are usually genetically-determined and mainly affect the kidneys or the liver (Figure 1). Their differential diagnosis, which is almost always based on imaging criteria, is crucial because polycystic kidney diseases include several clinical entities that have completely different symptoms, evolution and prognosis (1). Early diagnosis allows close supervision and management of kidney function and treatment of associated complications such as hypertension or nephrolithiasis that can delay the progression toward kidney failure (2).

Autosomal dominant polycystic kidney disease

Autosomal dominant polycystic kidney disease (ADPKD) is the most common form of monogenic, inherited kidney disease worldwide and affects 1 in 500 to 1000 individuals (3, 4). There are two known genes involved in ADPKD: PKD1 (discovered in 1994) which is

located on the short arm of the chromosome 16 and produces polycystin 1 (PC1), a transmembrane protein with a long N-terminal extracellular tail that can function as a mechanosensor; and PKD2 (discovered in 1996) which is located on the long arm of chromosome 4 and produces a smaller glycoprotein, polycystin 2 (PC2), that plays a role in calcium transport (3, 5, 6). A small group of all ADPKD families is not linked to either of the known genes, which suggests that there may be a third unknown gene (4). A few reports describe patients with ADPKD phenotype with no mutations in the two described genes (7-12), but some families which previously fit this group when re-evaluated showed a linkage to PKD1 or PKD2 mutations (13-15). Thus, the existence of a third gene for ADPKD is questionable at the present time (4, 6).

The PKD1 mutation accounts for 85% of ADPKD cases in clinically-identified populations, while PKD2 is responsible for the remaining 15% (3, 5, 6). Phenotypes associated with ADPKD show high levels of variability either in terms of age of onset of end-stage kidney disease (ESKD), associated liver disease or other extrarenal manifestations. This can be attributed to genic and allelic heterogeneity and environmental influences (3, 14). Regarding genetic heterogeneity, it is known that PKD1 mutations are associated with more severe disease, including earlier age at diagnosis, increased prevalence of hypertension and earlier onset of ESKD than mutations in PKD2 (median age: 54 vs 74 years old) (3, 4). At the allelic point of view, certain mutations are associated with more severe disease phenotype than others; for example, patients with truncating mutations have a more severe disease than patients with non-truncating mutations (4, 6). Most patients with ADPKD are reported to have truncating mutations (16). A study showed that

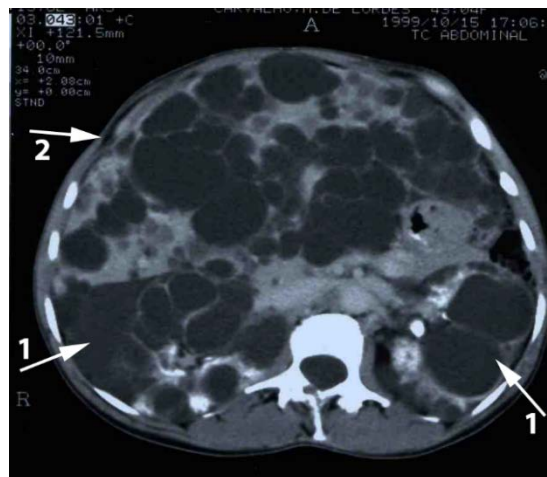


Figure 1. Polycystic kidney and liver disease. 1, Enlarged kidneys due to multiple cysts; 2, Enlarged liver with multiple cysts.

mutations located toward the 5' end of the PKD1 gene are associated with earlier onset of ESKD and increased risk of ruptured intracranial aneurysms (17).

Studies have suggested a gene dose-dependent effect on severity of ADPKD (3, 16). Complete penetrance homozygous mutations in PKD1 or PKD2 in humans are predicted to be embryonically-lethal (16). Although homozygous inheritance of incompletely penetrant PKD1 or PKD2 alleles can be associated with a more severe phenotype, heterozygous inheritance of the same alleles was associated with mild cystic disease (3, 16). The involvement of modifier genes (e.g. endothelial nitric oxide synthesis/ENOS and angiotensin converting enzyme/ACE) and epigenetic regulators (e.g. histone deacetylases/HDACs) may contribute to the complexity of phenotypic variability in ADPKD, since there are studies showing intrafamilial phenotypic variability in families where patients share the same mutation but show significant differences in disease severity and presentation (3).

Diagnosis of ADPKD

Despite constant increase in knowledge of the genetic features of this disease, the screening and diagnosis are based on imaging criteria according to age, family history and number of cysts in individuals (18-21). Ultrasound provides a cheap and safe method for diagnosis and screening of people with a high likelihood of ADPKD, but cost will prevent this screening from being applied to the whole population (6, 16, 22). This led McGovern et al. to identify clinical features that could be used in the early identification of people at high risk of ADPKD (22). The clinical features which best distinguished people with polycystic kidney disease were chronic kidney disease stage 3A or worse, proteinuria, haematuria, diastolic blood pressure (BP) greater than 90 mmHg, and being on multiple antihypertensive medications (22).

In the past the diagnostic criteria were based on patients who had a family history of PKD1. In 2009, the criteria were modified to include patients with a family history of PKD2 who began cyst development at a later age and with a lower number of cysts and at-risk adults of unknown gene type (Table 1) (4, 5, 6, 23). Accordingly, in younger subjects (15-29 years) at-risk because of an affected first-degree relative, three cysts in both kidneys, are sufficient for the diagnosis, whereas in older subjects where the finding of simple cysts is common, 4 or more cysts in both kidneys is required for the definite diagnosis of ADPKD. It is important to note that these unified criteria apply only to patients with positive family history of ADPKD, which excludes about 10-15% of possible ADPKD cases that do not have a family past of ADPKD (5, 6, 16). A positive family history can be absent due to new

mutations, mosaicism, mild disease from PKD2, non-truncating PKD1 mutations or unavailability of parental medical records (6). In this case, a patient with bilaterally enlarged kidneys and innumerable cysts, without other findings to suggest a different cystic disease (Table 2), most likely has ADPKD (6).

For older subjects, the presence of at least four cysts in each kidney is sufficient for diagnosis of ADPKD regardless of the gene type (4). On the other hand, a few patients who met the diagnostic criteria of ADPKD do not present the expected clinical features, like some degree of renal impairment or the known gene mutations (3, 24, 25). Beyond the most common renal phenotype, Van Gulick et al. described a subset of patients whose hepatic cysts are more prominent than renal cysts and who suffer more from their polycystic liver (> 20 cysts). Occasionally, some of ADPKD patients present with both renal and liver cysts, normal renal function and extensive hepatic disease (24, 25). Cnossen et al. identified the LRP5 gene as the third locus associated with isolated polycystic liver disease. As polycystic liver disease is the most common extrarenal feature in ADPKD patients, it was hypothesised that LRP5 variants may contribute to hepatic and renal disease heterogeneity in ADPKD. Although more studies are needed, it was postulated that LRP5 variants may render ADPKD patients more susceptible to the development of polycystic liver (14). These few cases where diagnosis is not linear means that doctors should be aware of other renal cyst diseases that need to be considered as a differential diagnosis of ADPKD. A substantial number of ADPKD patients progress to ESKD and this condition is responsible for 6-10% of patients in renal replacement therapies. Therefore, early diagnosis is important as treatment of associated complications such as hypertension or nephrolithiasis can delay the progression towards ESKD (2). Moreover, several drugs targeted to specific pathways that are altered in ADPKD were tested recently in randomized-controlled studies and will hopefully be available for human use in the near future (26).

Table 1. Unified criteria for ultrasound diagnosis of ADPKD (4, 5, 6, 23)

Age	Number of cysts	Sensitivity	Positive predictive value
15 – 29 years	A total of 3 cysts in both kidneys	81.7%	100%
30 – 39 years	A total of 3 cysts in both kidneys	95.5%	100%
40 – 59 years	2 or more cysts in each kidney	90%	100%

Table 2. Differential diagnosis of ADPKD (4, 6, 30-34, 36)

Condition	Gene	Inheritance	Prevalence	Differentiating signs/symptoms
Simple renal cysts	--	Acquired	Common	Normal renal function; normal-sized kidneys with smooth contour
Acquired renal cystic disease	--	Acquired	Common	Associated with CKD or ESKD; multiple renal cysts associated with small- to normal-sized kidneys
Medullary cystic kidney disease	MCKD1-2	AD	Unknown	Interstitial fibrosis on renal biopsy. Rarely cysts in the corticomedullary junction; slowly progressive renal failure; small- to normal-sized kidneys; Hyperuricemia, gout
Polycystic liver disease	PRKCSH / SEC63	AD	Unknown	Small number of renal cysts; predominantly liver cystic disease
Autosomal Recessive Polycystic kidney disease	PKHD1	AR	1: 20.000	Early in life kidneys cystic, enlarged and echogenic. With increasing age, kidneys are smaller with macroscopic cysts, nephrocalcinosis and/or small medullary calcifications common; Oligohydramnios (Potter's phenotype) and pulmonary hypoplasia in utero, congenital hepatic fibrosis, Caroli's disease
Tuberous Sclerosis	TSC 1-2	AD	1: 10.000	Angiomyolipoma. Contiguous deletion of PKD1/TSC2 results in severe early onset PKD with ESKD typically occurring in the first 2 decades of life. Skin lesions (facial angiofibromas, periungal fibroma, hypomelanotic macules and Shagreen

ADPKD – subtypes and differential diagnosis

				patch); retinal hamartomas; seizures; mental retardation; cortical tuber; subependymal giant cell astrocytoma; cardiac rhabdomyoma; lymphangioleiomyomatosis
Von Hippel-Lindau	VHL	AD	1:50.000	High risk of renal cell carcinomas. CNS and retinal hemangioblastomas, pancreatic cysts, pancreatic endocrine tumours, pheochromocytoma
Orofaciodigital syndrome I	OFD1	X-linked, dominant	Very rare (1:250.000)	Embryonic male lethal, cleft palate, bifid tongue, hyperplastic frenula, hypertelorism, broadened nasal ridge, digital abnormalities including syndactyly, CNS malformations
Nephronophthisis	NPHP1-6	AR	1:10.000	Normal-sized kidneys with corticomedullary junction cysts; Interstitial fibrosis; Retinitis pigmentosa; Cerebellar vermis aplasia, polydactyly, occipital encephalocele (NPHP 1 / 6); ocular motor apraxia (NPHP 1-2); liver fibrosis (NPHP2-3), situs inversus (NPHP 2)
Bardet-Biedl syndrome	BBS 1-12	AR	1:140.000	retinal degeneration, childhood obesity, mental retardation, malformations of the urogenital tract, polydactyly
Medullary sponge kidney	Unknown	Unknown	1:5000	Malformation of the distal collecting tubules with nephrolithiasis (haematuria), renal

				function impairment, tubular acidosis, recurrent urinary tract infections
Localised cystic disease	--	Unknown	Unknown (very rare)	Benign disease, unilateral cysts, no progression to chronic renal failure, no extrarenal involvement
Meckel-Gruber syndrome	MGS 1-6	AR	1:13,250 to 1:140,000	Occipital encephalocele, and postaxial polydactyly
Renal cysts and diabetes syndrome	HNF1B	AD	Unknown	Renal malformation, diabetes mellitus, hypomagnesemia, genital tract abnormalities, hyperuricemia and elevated liver enzymes

AD, autosomal dominant; AR, autosomal recessive; CKD, chronic kidney disease; ESKD, end stage kidney disease; CNS, cerebral nervous system.

Direct molecular genetic tests for diagnosis

In most circumstances, diagnosis of ADPKD is based on clinical grounds: history, particularly family history; physical examination; and renal ultrasound. Confirmation of the diagnosis will occur in follow-up studies when an increasing number and size of the renal cysts is evident as well as the appearance of hepatic cysts, hypertension, urologic symptoms and cardiovascular disease. So far genetic studies are not easily available for clinical diagnosis because they are expensive and add no advantage for clinical follow-up (although we expect that this will change with the evidence that there is a correlation between the type of mutation and the prognosis of the disease) (27). Moreover this analysis can be complicated as a result of the duplication of the 1-32 exons of PKD1 gene as pseudogenes in chromosome 16 and the high level of allelic heterogeneity. In general no mutation was identified in about 8% of the families that participated in the HALT-PKD trial (28). Therefore, genetic studies are reserved for a limited number of situations like living-related donors of renal transplant, to exclude the diagnosis of ADPKD in the donor, for pre-natal counselling particularly in the pre-implantation in vitro fertilization and to resolve complex renal cystic disease, if clinically important (27).

Which diagnosis should doctors consider when the patient has multiple renal cysts?

In a majority of cases, establishing a diagnosis of ADPKD is simple. The typical patient presents with enlarged cystic kidneys in the setting of a positive family history (4).

However cystic kidneys in absence of a family history of ADPKD requires a careful review of the history looking for clinical and radiologic aspects that may reveal clinical features of these disorders that are atypical of ADPKD. Table 2 summarises the differential diagnosis of ADPKD.

Renal cysts are common in adults. Finding renal cysts in an ultrasound in an asymptomatic subject raises several options for the diagnosis like simple cysts, ADPKD or acquired renal cysts. Careful history and physical examination, as well as, other clinical findings such as hypertension, liver cysts or renal failure facilitate the differential diagnosis between these common entities. However, in certain circumstances, particularly with atypical clinical presentations the following entities should be excluded:

- *Multiple benign simple cysts:* Prevalence of simple renal cysts increases with age and they are commonly detected with sensitive imaging methods such as computed tomography (CT) or magnetic resonance imaging (MRI). MRI-based series detect at least one renal cyst in 93% of subjects 45-59 years of age (4). Spiral CT detected simple renal cysts in 41% of 617 patients. Renal cysts are present in approximately 50% of men (mean age 66 years) and 35% of women (mean age 63 years) (27, 29-30).
- *Localised renal cystic disease:* Localised renal cystic disease is a rare and benign condition. Patients may present hypertension, flank pain, haematuria or flank mass. Clinical features distinguish this disease from ADPKD because of unilateral location, negative family history, no progression to chronic renal failure and no extrarenal involvement (29).
- *Acquired renal cystic disease:* Acquired renal cystic disease is typically detected in individuals with longstanding renal failure and can be found in up to 20% of patients with ESKD (4). The main distinction is the presence of cysts in a normal or small kidney with signs of chronic kidney disease (4, 6).
- *Medullary sponge kidney:* Medullary sponge kidney is a cystic renal disease with unknown inheritance characterised by malformation of the distal collecting tubules with nephrolithiasis, impairment of renal function, tubular acidosis and recurrent urinary tract infections – but rarely evolves to ESKD (32).
- *Bilateral parapelvic cysts:* Parapelvic cysts are a subset of simple cysts that arise within the renal parenchyma adjacent to the renal sinus and account for 5% of all

renal cysts in adults. Clinically they may manifest through obstruction of the ureter or renal pelvis (33).

- *Autosomal recessive polycystic kidney disease:* ARPKD affects 1 in 20000 births and causes neonatal deaths in 30% of patients (6). As the result of a mutation in the PKHD1 gene (31) on chromosome 6, patients usually present in the neonatal period with enlarged, echogenic kidneys with occasional cortical cysts (4, 32). Other perinatal manifestations include Potter's phenotype, pulmonary hypoplasia and portal fibrosis (6, 32). ARPKD is commonly diagnosed at an early age, but adult forms have been described (4). Distinguishing features include bilateral large echogenic kidneys with poor differentiation but, in contrast with ADPKD, with few macrocysts, absence of liver cystic disease but with presence of congenital hepatic fibrosis and/or Caroli's disease (4).
- *Tuberous sclerosis complex:* Tuberous sclerosis complex is an autosomal dominant disease, with incidence 1:5000 to 10 000 and is caused by mutations of the TSC1 or TSC2 gene (4, 32). Family history of the disease is absent in two-thirds of families (6). Mutations of TSC2, which is located tail-to-tail with the PKD1 gene in the short arm of chromosome 16, may result in polycystic renal changes resembling ADPKD, particularly in the so-called contiguous gene syndrome (32). Angiomyolipomas of the kidneys, facial angiofibromas, retinal hamartomas, cerebral pathology (cortical tuber and subependymal giant cell astrocytoma) and benign neurocutaneous tumours allows differentiation (4, 6, 32).
- *Von Hippel-Lindau syndrome:* Autosomal dominant-inherited von Hippel-Lindau (VHL) syndrome is characterised by a combination of hemangioblastomas (retina and cerebellum), renal cell cancers and, less frequently pancreatic, endocrine tumours and pheochromocytoma (4, 32). In the early stage, precancerous renal cysts may occur, which result in enlargement of the kidneys and may be misdiagnosed as ADPKD. However, kidney failure is not a major feature in VHL syndrome (32).
- *Autosomal dominant medullary cystic disease:* Autosomal dominant medullary cystic disease may be present in adulthood (30–60 years) with renal dysfunction and, occasionally, renal cysts (4, 32). The gene involved is uromodulin (encoding Tamm-Horsfall protein) on chromosome 16. Distinguished features are tubular interstitial fibrosis with normal- to small-sized kidneys usually accompanied by early hyperuricemia and gout (4).

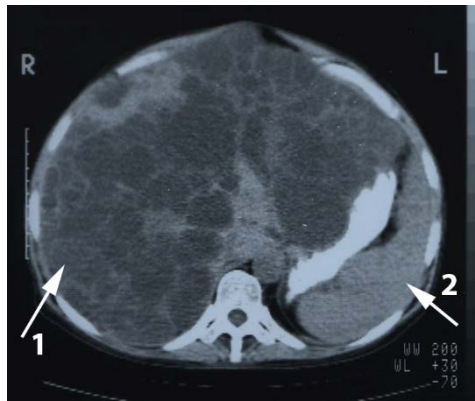


Figure 2. Polycystic liver disease. 1, Enlarged liver due to uncountable cysts; 2, Spleen.

- *Autosomal dominant polycystic liver disease:* Autosomal dominant polycystic liver disease is easily mistaken for mild ADPKD, with the main difference being the absence of cystic kidney disease. However, these patients may have few renal cysts leading the clinician to consider a diagnosis of ADPKD, but as a general rule these individuals have predominantly polycystic liver disease and do not develop ESKD (Figure 2) (4). As previously discussed, very few patients with ADPKD present a predominant liver cyst phenotype. When the cysts are also present in the kidneys, the number of liver cysts is small, and a family history of polycystic liver disease or ADPKD is absent, it may be impossible to distinguish the two diseases. Under these circumstances, clinical follow-up and genetic studies may be helpful (34).
- *Orofaciodigital syndrome type I:* This is an X-linked disease due to mutation of the OFD1 gene, and craniofacial and digital defects are associated with polycystic kidney disease (32, 35).
- *Bardet-Biedl syndrome:* Bardet-Biedl syndrome is a very rare disease with incidence 1:140,000, due to diverse mutations in genes coding for proteins involved in the primary cilium functions which entitled this condition as a ciliopathy. Polycystic kidney disease coexists with several extrarenal defects, such as vision loss due to retinal degeneration, childhood obesity, mental retardation, malformation of the urogenital tract and polydactyly (32).
- *Renal cysts and diabetes syndrome:* Type 5 MODY (Maturity Onset Diabetes of the Young) is a rare monogenic disease resulting from mutations in hepatocyte

nuclear factor 1 β (HNF-1 β) that is associated with renal cysts. About 50% of this autosomal-dominant disease results from de novo mutations, and presents with renal cysts or malformation in 90% of patients. It also presents with diabetes mellitus in 45% of patients, genital tract abnormalities and hyperuricemia in 20%, hypomagnesaemia in 40% and elevated liver enzymes in 15% (6).

In children with the finding of renal cysts in absence of a family history of ADPKD, the differential diagnosis should consider the following entities:

- *Autosomal Recessive Polycystic Kidney Disease (ARPKD)*: See detail above.
- *Contiguous PKD1-TSC2 syndrome*: This is a syndrome that results from deletions in the short-arm of chromosome 16 involving both PKD1 and TSC2 genes. These patients usually present in infancy with features of tuberous sclerosis and polycystic kidneys and rapid progression to ESKD. Frequently there is no family history, since the parents are somatic mosaics or results from de novo mutations (4).
- *Meckel-Gruber syndrome*: This is a rare autosomal recessive lethal malformation, characterised by the triad occipital encephalocele, bilateral polycystic kidneys and post axial polydactyly (36).
- *Nephronophthisis*: This condition is characterised by the formation of cysts at the corticomedullary junction without enlargement of the kidneys and generally leads to ESKD before the second decade of life. Numerous extrarenal manifestations are seen, such as Retinitis Pigmentosa, cerebellar ataxia, oculomotor apraxia and hepatomegaly. Six different gene mutations induces this autosomal recessive disease (NPHP1 to NPHP6) a major cause of renal failure in children (32).

Conclusion

ADPKD is a common disease with an important impact on the quality of life and survival of many patients across the world, accounting for 6-10% of patients on renal replacement therapy, making it a burden to families and society. The knowledge about genetic heterogeneity and phenotypic variability is essential for the correct diagnosis and classification. Studies on the genetic development and understanding of the ADPKD pathogenesis have shown an increasing trend, allowing for clarification of the diversity

of clinical and genetic aspects of many patients who do not present classic frames or even the variability within families in patients with the same genetic defect. There are several other disorders that may mimic ADPKD; however, in most cases, additional findings allow an easy differential diagnosis. Atypical ADPKD patients should undergo further evaluation, since management, evolution and prognosis may differ among these disorders.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 2

Childhood Polycystic Kidney Disease

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Abstract

Autosomal recessive polycystic kidney disease (ARPKD), historically called infantile PKD, is a major cause of morbidity and mortality in neonates, infants and young adults. Autosomal dominant polycystic kidney disease (ADPKD), historically referred to as adult PKD, is increasingly recognized as a significant cause of morbidity and mortality in children and young adults. ARPKD, a dual-organ disease with hepatic and renal

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involvement has an incidence of 1: 20,000 to 1: 40,000. All ARPKD patients are invariably afflicted with congenital hepatic fibrosis (CHF) of varying degrees of severity. Improved survival of ARPKD patients has led to recognition of significant clinical complications of CHF and the highly variable age at which it presents, ranging from early childhood to young adulthood. ADPKD, with an incidence as high as 1:400, affects more than 13 million individuals worldwide, and accounts for 7-10% of end stage kidney disease (ESKD) in adults. However, asymptomatic disease is increasingly recognized in infants and children and nearly equivalent numbers of ADPKD and ARPKD patients may be seen in academic pediatric nephrology clinics. The delineation of the basic molecular and cellular pathophysiology of ADPKD and ARPKD has seen remarkable progress in the last decade. This progress has led to the development of promising therapies currently being evaluated in clinical trials. Early diagnosis of ADPKD and ARPKD allows for optimal anticipatory care (for example, early blood pressure control). Given the predicted benefit of early intervention with new disease-specific therapeutics, screening at-risk youth, a previously-discouraged strategy, may now be warranted. This chapter will discuss central clinical characteristics essential for diagnosis and the care of children with ARPKD or ADPKD. We will also highlight recent insights in the molecular and cellular pathophysiology of PKD and the clinical translation into new therapies that promise to alter the natural history of disease for children with genetic PKD.

Key words: BP control; CHF; Combination therapy; c-Src; EGFR; Fibrosis; Preemptive care

Introduction

Cystic kidney diseases (CKDs) are a clinically and genetically diverse group of renal cystic diseases that have tubular cysts or renal dysplasia as a phenotypic element of their disease phenotype (1). These “phenocopies” and differentiating features are covered in detail in different Chapters in this text. Traditionally, the term polycystic kidney disease (PKD) refers to one of two genetically-distinct disorders: autosomal recessive polycystic kidney disease (ARPKD-OMIM 263200); and autosomal dominant polycystic kidney disease (ADPKD-OMIM 173900 and OMIM 173910).

ARPKD is the result of mutations in a single gene, the polycystic kidney and hepatic disease 1 (PKHD1) gene which encodes the fibrocystin/polyductin protein complex (FPC). ARPKD is typically diagnosed in the latter half of the trimester of pregnancy at birth or shortly thereafter with massive bilaterally enlarged kidneys that may complicate delivery. This disease was traditionally called infantile PKD, a name that no longer applies.

ADPKD is a heterogenic disease caused by mutations in two genes, PKD1 encoding polycystin 1 (PC1) and PKD2 encoding polycystin 2 (PC2). ADPKD, usually asymptomatic well into adulthood, is characterized by bilateral, progressive growth of renal cysts that most likely began *in utero*. It is now recognized that that both ARPKD and ADPKD can be clinically symptomatic in infants, children and adolescents and causing substantial degrees of morbidity and mortality in these age groups (2-8). The manifestations of childhood PKD can have considerable significant overlap in clinical and radiographic features making differential diagnosis between the two diseases difficult. This chapter will highlight the central clinical characteristics essential for the diagnosis and care of children with ARPKD or ADPKD, followed by a discussion regarding the existing knowledge of the molecular and cellular pathophysiology of these two genetic diseases, and how this information is informing development of current and future PKD-specific therapies.

Although ADPKD is typically an adult-onset, systemic disease, when presenting in the neonatal period, ADPKD may be clinically indistinguishable from ARPKD (9-11). In such instances, a detailed history, complete physical, imaging, and rarely, genetic testing and/or biopsy are usually sufficient to distinguish the two (7, 11, 12). Although no single finding is diagnostic (4, 6, 11) certain clinical features can help differentiate between ARPKD and ADPKD and these differentiating features are discussed in detail in different Chapters. Early diagnosis of asymptomatic individuals with either ARPKD or ADPKD currently offers the opportunity for maximal preemptive care such as tight blood pressure control, close surveillance of extrarenal manifestations (CHF, cardiovascular disease), avoidance of potential dietary and lifestyle progression factors and targeted disease-specific therapies that are on the horizon (6-8, 11, 13,14).

Autosomal recessive polycystic kidney disease (ARPKD)

ARPKD (OMIM 263200) is a rare, hepatorenal fibrocystic disease, characterized by non-obstructive, fusiform, cystic distension of renal collecting ducts with unpredictable degrees of congenital hepatic fibrosis (CHF), as a result of a biliary ductal plate malformation. Progressive biliary disease leads to periportal fibrosis or CHF, and may progress to Caroli disease, a cystic widening of the intrahepatic bile ducts; and the common bile duct (4, 6 , 12, 15, 16). ARPKD has an incidence rate of 1/20,000, is commonly diagnosed in utero or at birth, and despite a wide variability in phenotype, occurs due to mutations in a single gene, the PKHD1 gene (17-19). Despite the characteristic neonatal presentation of ARPKD, there is a significant variability in both age and presentation of initial clinical symptoms including the comparative level of renal and biliary abnormalities (15, 20, 21). The variability in the degree of organ involvement in ARPKD is not well understood but it is

generally recognized (7, 22-24). This phenotypic variability is regulated by the influence of other disease modifying genes, the combination of the two different parental PKHD1 mutations, epigenetic factors, hormonal effects, and environmental influences (23, 25).

Epidemiology and genetics

ARPKD is caused by mutations of PKHD1 gene that encodes the FPC. PKHD1 was cloned by two independent research groups in 2002 (18, 19). PKHD1 is an exceptionally large gene that spans approximately 470 kb of genomic DNA and consists of 86 exons, with 67 exons included in the longest open-reading frame transcript (18). A number of alternatively spliced transcripts have been identified; however, the exact function and clinical significance of these isoforms are unknown (26). Nearly 750 PKHD1 mutations have been identified to date (<http://www.humgen.rwth-aachen.de>).

With the cloning and identification of PKHD1 as the causative gene in ARPKD, attempts to define genotype-phenotype correlations have been attempted but to date only general correlations can be made. Recent discoveries regarding transcriptional complexities have only complicated any tenuous correlations and must be considered for appropriate genetic counseling.

A series of published ARPKD kinships demonstrate that mutations in PKHD1 are dispersed across the gene, without obvious clustering at mutational hotspots, and most families have distinctive ("private") mutations (19, 27-29). Additionally, nearly all patients are compound heterozygotes (23, 30), that is, each allele carries a different mutation. Missense, nonsense, frame shift and truncating mutations have all been described (31).

Patients with truncating mutations on both alleles typically displayed a severe phenotype, with a high rate of perinatal or neonatal demise (23, 27, 30). Patients with a least one missense mutation (amino acid substitutions) are more likely to survive the neonatal period and beyond (32, 33). Boddu et al. found 22 novel renal transcripts derived through numerous methods of alternative splicing. These include the use of alternate acceptor/donor splice sites, exon skipping and in a specific animal model of Pkhd1, inclusion of novel exons (26). These studies suggest that unconventional PKHD1 splicing occurs frequently and these splicing events may represent another pathogenic mechanism leading to ARPKD (26). The functional activity of these transcripts is unknown but these observations add a level of complexity for genetic counseling.

As the term autosomal recessive inheritance indicates, parents are heterozygotes (carriers), and are clinically-unaffected. The risk of any pregnancy producing an affected offspring is

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25% and there is a 50% chance that unaffected offspring will carry a mutation in PKHD1 (4, 12). There appears to be no gender preference for ARPKD and all ethnic and racial groups are equally affected.

Diagnosis

With the advent of modern obstetrical ultrasonography (US), many patients with ARPKD are identified in the prenatal period. The diagnosis of ARPKD is based on clinical observations and our current understanding of the disease process. The diagnostic criteria proposed by Zerres et al. with modifications (11, 12, 34), are generally used and include:

1. US features typical of ARPKD, including enlarged, echogenic kidneys, with poor corticomedullary differentiation; and
2. One or more of the following:
 - a. Absence of renal cysts in both parents, particularly if they are at least 30 years old,
 - b. Clinical, laboratory or radiographic evidence of hepatic fibrosis,
 - c. Hepatic pathology demonstrating characteristic ductal plate abnormalities,
 - d. Previous affected sibling with pathologically or genetically confirmed disease,
 - e. Parental consanguinity suggestive of autosomal recessive inheritance.

ARPKD is typically diagnosed in utero or at birth and manifests as progressive renal insufficiency and portal hypertension (PH) (34-36). The most severely affected fetuses display a classic "Potter" phenotype (oligohydramnios sequence) with massively enlarged echogenic kidneys with poor corticomedullary differentiation due to fusiform dilatation of the collecting ducts, respiratory insufficiency, cranial abnormalities and club feet (36). Oligohydramnios is detectable in utero, but may not develop in ARPKD until the third trimester (37).

Pulmonary insufficiency, a serious complication that generally occurs as the result of oligohydramnios, results in respiratory failure and subsequently neonatal death in approximately one third of neonates presenting with large, echogenic kidneys (4, 7, 11, 38). Patients that survive the neonatal period have a 1 year survival rate of 85% and a 10 year survival rate of 82% (25, 36). A smaller, though increasingly recognized subset of patients with ARPKD are diagnosed as older children or adults with abdominal enlargement secondary to enlarged kidneys or hepatosplenomegaly (21, 35, 39). These patients typically present with signs and symptoms related to congenital hepatic fibrosis including PH and esophageal bleeding (20, 25, 40). Other associated comorbidities including systemic hypertension, progressive renal insufficiency, and less commonly,

chronic lung disease in older children (35), especially in children who had respiratory insufficiency at birth. Improvements in neonatal critical care have allowed neonatal survival rates to improve, and with more patients with ARPKD surviving to adulthood, liver and other complications are likely to become more prevalent (20).

Pre-implantation genetic diagnosis through linkage analysis or mutational analysis is possible in families with at least one pathologically or genetically confirmed affected child when the mutation on both the maternal and paternal allele can be identified. Linkage analysis can also be used to identify the carrier status of an unaffected child. In “genetically informative” families, the accuracy of prenatal diagnosis using linkage analysis is >95% (41). Successful preimplantation genetic diagnosis has been reported for ARPKD (42). A list of laboratories that offer genetic testing including pre-implantation genetic diagnosis for ARPKD is available at www.geneclinics.org.

Pathology

The typical kidney phenotype consists of enlarged echogenic kidneys with loss of corticomedullary differentiation due to fusiform dilatation of the collecting ducts. Renal ultrasound of infants and young children, reveal bilaterally enlarged echogenic kidneys with poor corticomedullary differentiation (Figure 1a). The kidneys retained a reniform contour (Figure 1b), and multiple tiny cysts are confined to collecting ducts (Figure 1c) (11, 12, 36, 43). Macroscopically, the cut surface reveals a radial pattern of the spindle-shaped collecting duct cysts that extend into the renal cortex (Figure 1c). Microscopically (Figure 1d) the cysts are usually less than 2 mm in diameter and microdissection and immunohistological studies have demonstrated these “microcysts” to be dilated collecting ducts (36, 43-45). The glomeruli and other tubular segments appear to be decreased in number due to collecting duct ectasia and interstitial changes that squeeze and atrophy the renal parenchyma. Proximal tubular cysts have been identified in fetal kidneys, (46), but are generally not seen after birth. The calyces, renal pelvis and renal vessels appear normal.

In contrast to renal cysts in ADPKD, where the tubular cysts detach from the tubule of origin, the fusiform cystic tubules in ARPKD remain in contact with the urinary stream. This has two important implications: 1) obstruction is not a component of cyst formation in ARPKD (44, 47); and 2) the afferent and efferent opening of an ARPKD collecting duct cyst remains in continuity with the urinary stream, and thus the urine is more likely to reflect changes that occur with cystic formation and growth. In ADPKD, where cysts pinch off from the tubular segment of origin, changes occurring in the cystic lesion are unlikely to be reflected in the urine. With increased patient survival and disease progression, hyperplasia results in larger renal cysts and interstitial fibrosis begins to develop which produces a pattern more like ADPKD (see Figure 3) (11, 12, 48).

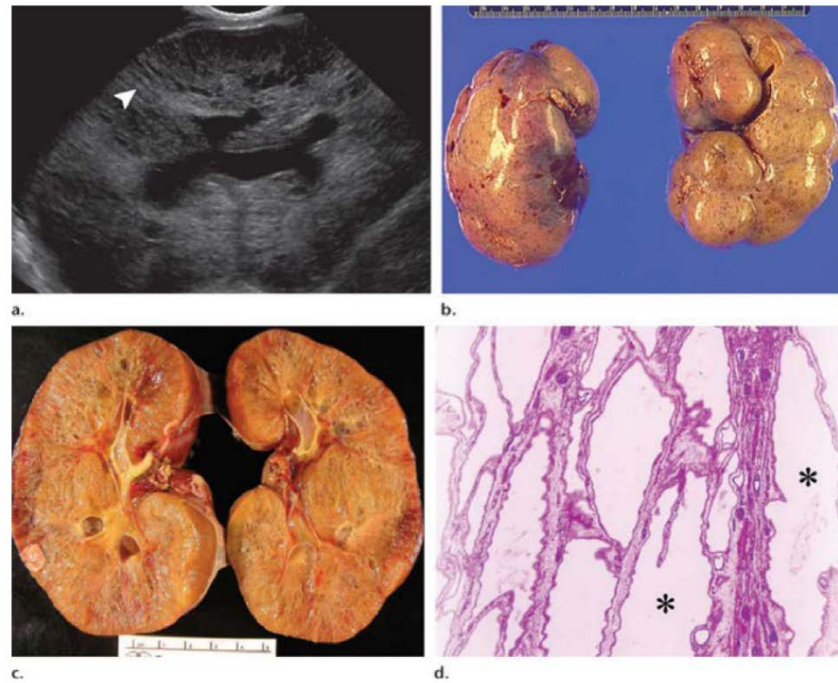


Figure 1. ARPKD in a 2-month-old boy. (a) Longitudinal high-spatial-resolution (8-MHz) ultrasonographic (US) image of the right kidney shows diffuse tubular ectasia involving the medulla and extending to the cortical surface (arrowhead). Note that the tubular nature of these cysts can be resolved by using a high-frequency transducer. The left kidney demonstrated a similar appearance (not shown). (b) Photograph of the gross specimen of the kidneys reveals a spongy appearance of the surface due to underlying cysts involving the cortex. (Scale is in centimeters.) (c) Photograph of the bivalved kidney shows a streaky appearance due to dilated tubules extending to the cortical surface with obscuration of the corticomedullary junction. (d) Photomicrograph (original magnification, $\times 4$; hematoxylin-eosin stain) of the kidney shows the radially-oriented, ectatic collecting ducts (*) with glomeruli and normal tubules between the cysts, perpendicular to the connective tissue capsule. Reprinted, with permission, from Chung, et al. *RadioGraphics* 2014; 34:155-178 (43).

Biliary ectasia and the consequential hepatic fibrosis are invariably present in ARPKD. These biliary abnormalities are the result of a ductal plate malformation resulting in congenital hepatic fibrosis and biliary ductal ectasia (Figure 2) (7, 12, 16, 20). Although hepatic involvement is invariably present at the microscopic level at birth, it is symptomatic in only 40-50% of neonates (34). As the CHF advances, hepatomegaly and PH develop in a number of patients.

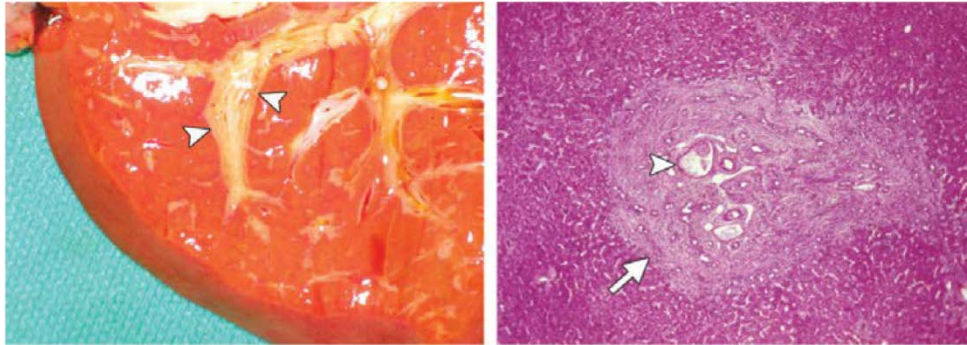


Figure 2. Pathologic findings of the liver in ARPKD-CHF. (a) Photograph of a liver section shows periportal fibrosis (arrowheads). (b) Photomicrograph (original magnification, $\times 10$; hematoxylin-eosin stain) of the liver shows the portal vein and hepatic artery (arrowhead) in a portal area expanded by fibroblastic proliferation (arrow). Reprinted, with permission, from Chung, et al. *RadioGraphics* 2014; 34:155-178 (43).

PH is an increase in the blood pressure within the portal venous system and is clinically defined by the presence of splenomegaly, thrombocytopenia, low platelets counts, hypersplenism, enlarged hemorrhoids, and esophageal and gastric varices (20). In ARPKD, intrahepatic dilatation of both the central bile ducts and both large and small peripheral bile ducts in the setting of CHF (Caroli's syndrome) can occasionally progress to macrocysts and dilation of the extrahepatic bile duct (12, 20, 49). The combination of renal collecting duct and biliary ectasia with periportal fibrosis is unique to ARPKD (16, 40, 50-52).

Clinical

Lung development requires amniotic fluid to distend the developing fetal lung and adequate physical space for diaphragmatic movement is required (38). In ARPKD, oligohydramnios and restriction of diaphragmatic movement due to large cystic kidneys results in pulmonary hypoplasia. Pulmonary insufficiency is the major cause of morbidity and mortality in neonates with ARPKD. Infants with true pulmonary hypoplasia remain hypoxemic despite neonatal interventions and rarely survive (4, 11, 12).

At birth, patients usually have large, palpable flank masses that may be large enough to complicate delivery. Most newborns with ARPKD (70-80%) have some degree of impaired renal function which is often followed by a brief improvement as respiratory issues are alleviated, and renal development continues (7, 10, 34). Hyponatremia which may exist initially, usually resolves quickly, unless the patient has acute renal failure (34, 53). Most patients have a urinary concentrating defect and symptoms of polyuria and polydipsia (7,

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10, 12, 48, 53, 54). Although kidneys may be markedly enlarged at birth, over time, the majority show stable to decreased renal size (12, 55, 56).

Although all ARPKD patients demonstrate some degree of microscopic evidence of CHF at birth, the consequence of these findings is unpredictable. Complications of CHF may develop at any time between birth and adulthood or they can remain asymptomatic even into adulthood (57). The renal and hepatobiliary disease components of ARPKD advance at varying rates for reasons that are unclear. However, the progressive biliary ductal ectasia and CHF must be closely monitored because the hepatobiliary disease can lead to the development of PH which can be severe (12, 16, 20).

PH is the main complication of CHF. Manifestations of PH can include hepatosplenomegaly, hypersplenism with low platelet counts, variceal and hemorrhoid bleeding and most importantly an increased risk of developing bacteremic infections from ascending cholangitis (7, 11, 16, 57). Recent studies of PH in ARPKD reveal that this progression starts early in life, and can initially be identified by hepatomegaly that starts in the left lobe of the liver (20, 34).

The clinical significance of severe hepatobiliary disease has become an increasingly important as ARPKD survival rates improve. Recent studies into the hepatobiliary component of ARPKD estimate that 40% of ARPKD survivors will demonstrate severe renal and severe hepatobiliary disease. The remaining 60% of ARPKD survivors will exhibit degrees of dual-organ disease that can be generally grouped into either severe kidney/mild hepatobiliary disease, mild kidney/severe hepatobiliary disease, or mild kidney/mild hepatobiliary disease (20, 58). Platelet count was identified as an accurate measure of the severity of PH in patients with ARPKD and correlates well with spleen size (20).

Hypertension, a common presenting feature in both infants and children, is often severe, can be difficult to control and frequently requires multidrug treatment (6, 11, 12, 39, 48, 59). Hypertension is often found in patients with normal renal function and it will eventually affect nearly all children with ARPKD (11, 24, 35). The pathophysiology of hypertension in ARPKD multi-factorial but activation of the intrarenal renin-angiotensin system is thought to be an important component (15).

Treatment and complications

Advances in neonatal critical care have led to increased survival rates of neonates with ARPKD. Predicting which neonates with ARPKD who require immediate artificial

ventilation will have life-threatening degrees of pulmonary hypoplasia is currently not possible (4, 7, 10). This is due to the fact that, severe pulmonary distress may be caused by a potentially-reversible fluid overload, true neonatal pulmonary hypoplasia, or restricted motion of the diaphragm due to massively enlarged kidneys. In selected cases, a more accurate assessment of the long-term pulmonary prognosis of the patient can be achieved through continuous venovenous hemofiltration, or a unilateral or bilateral nephrectomy to allow diaphragmatic movement, coupled with peritoneal dialysis (60, 61).

Young patients with ARPKD, including those without significant renal insufficiency, should be closely monitored. The loss of concentrating ability in these patients creates a significant risk of dehydration with intercurrent illnesses. Hyponatremia due to impaired urinary dilution (rather than sodium wasting) is common in ARPKD. When present, fluid intake should be curtailed without compromising nutrition (24). In patients with severe polyuria, thiazide diuretics may be used to decrease distal nephron solute and water delivery. Patients with metabolic acidosis will require supplemental bicarbonate therapy.

Urinary tract infection (UTI) rates as high as 50% have been reported with in patients with ARPKD, with girls having a greater frequency than boys (12). Any child with an abnormal urinalysis, antibody therapy should be guided by clinical features and appropriately obtained urine cultures. If a UTI is diagnosed, an evaluation to rule out vesico-ureteral reflux, obstruction, or bladder dysfunction is recommended (4, 7, 62). Infants and children with ARPKD are at risk of the consequences of progressive CKD (e.g. growth failure, anemia, renal osteodystrophy and cognitive deficits) and this risk increases as renal function declines (7, 11, 12).

In patients with symptomatic end stage kidney disease (ESKD), peritoneal dialysis is preferred modality and is shown to work well even with intra-abdominal organomegalies including liver, spleen and kidneys [9, 40]. Renal transplant should be appropriately considered (7, 24). If transplantation is necessary, a nephrectomy may be necessary to permit room for transplant placement in patients with enormous kidneys and may be indicated to control hypertension. Patients suffering from severe renal and severe biliary disease should be evaluated for a combined liver and kidney transplant (58). A decision tree that guides appropriate choices has been developed and can be found in Telega et al. (58).

Difficulties in feeding and poor growth, even in patients without renal insufficiency, are often noted. In such cases, regular nutritional evaluation, can help guide appropriate therapy, and feeding specialists should be part of the multidisciplinary care team. Data from the NIH-supported Natural History Study of ARPKD indicate that normal growth curves can be achieved with appropriate support measures (51). Improvements in neonatal

critical care and advances in renal replacement therapy have improved patient survival and as a result hepatic complications become the dominant clinical issue of many patients with ARPKD (20, 34, 35, 63-65). PH, an increase in the portal venous pressure occurs in 37-65% of ARPKD neonatal survivors (20, 25, 35). Despite the development of PH, hepatocellular function frequently remains normal (11, 16, 20).

The most serious complications of PH are bacterial cholangitis and bleeding varices. ARPKD patients with extensive dilatations of intrahepatic and extrahepatic bile ducts are at increased risk of ascending bacterial cholangitis. Development of fever or rarely a sudden elevation of liver function tests at any time should raise the suspicion of cholangitis and appropriate evaluation and antimicrobial therapy should be initiated.

From the time of initial diagnosis, all patients with ARPKD should be evaluated by a gastroenterologist and undergo regular evaluations for hepatobiliary complications (58). Recommended evaluations should include periodic imaging by either magnetic resonance cholangiopancreatography (MRCP), magnetic resonance imaging (MRI) or ultrasound on an annual basis for increased liver echogenicity, hepatobiliary abnormalities, and splenomegaly. Platelet counts have been reported to be a surrogate marker of the severity of PH and should be checked immediately (20). Endoscopy is routinely required to find varices early and treat them by endoscopic band ligation (EBL) to prevent potentially lethal bleeding. Platelet count (as a surrogate marker of the severity of PH) (20) and hemoglobin/hematocrit are routinely monitored as a sign of hypersplenism, splenic sequestration and GI hemorrhage with varices. Porto-systemic shunting may be indicated in cases where variceal sclerotherapy is inappropriate or has failed (53, 66), but Telega et al. recommend this should be done in consultation with a transplant surgeon (58). Patients with severe PH and moderate to severe renal disease should be evaluated for dual liver and kidney transplant (58).

ARPKD patients are potentially at risk for neurocognitive and behavioral dysfunction due to early onset severe hypertension and CKD (67) although little data exist to guide clinical practice (24). In addition to the significant medical problems, the psychosocial stresses of ARPKD on the patient and family can be formidable (4, 7) and should be kept in mind. Social support measures may be required for exhaustion because of due to the demands of new roles, depleted finances, and other aspects of a changed lifestyle (4).

A multi-disciplinary team that includes a pediatric nephrologist, and pediatric gastroenterologists in concert with, dietitians, social workers, and if needed psychiatrists and other support staff may be necessary to provide optimal comprehensive care for patients with ARPKD.

Autosomal dominant polycystic kidney disease (ADPKD)

ADPKD, more common than ARPKD, has a incidence rate of 1:400-1:1000 with 13 million people affected worldwide, and accounts for 7 to 10% of ESKD in adults. ADPKD was historically termed “adult” polycystic kidney disease due to the classic presentation of clinical symptoms that typically do not arise until adulthood. ADPKD is a systemic disease with early-onset hypertension and extrarenal manifestations that include, cystic lesions in the liver, spleen, and pancreas and vascular abnormalities that may including mitral valve prolapse and less frequently, intracranial aneurysms (ICAs).

Despite the classic textbook description of ADPKD as an adult disease, significant clinical manifestations of ADPKD can be seen in children and in some cases, in utero (2, 3, 5, 8). Pediatric patients who do present in childhood have similar renal findings to those of affected adults. In contrast, the extrarenal manifestations of ADPKD commonly seen in adults (for example, cysts in the liver and pancreas and ICAs) are infrequently or rarely observed in pediatric patients. A detailed discussion of these extrarenal manifestations of ADPKD can be found in other Chapters of this volume and therefore only pediatric specific topics stemming from childhood ADPKD will be discussed below.

It is increasingly being recognized that pediatric ADPKD patients, with early manifestations of disease, are most likely to benefit from early therapeutic interventions (7, 8, 11, 68). It is important therefore that clinicians be aware of these ADPKD-related manifestations when caring for children with, or at risk of, ADPKD.

Renal cysts in ADPKD kidneys form in utero and bilateral enlarged cystic kidneys, left ventricular hypertrophy with or without systemic hypertension, proteinuria, gross hematuria, nephrolithiasis, flank pain, and impaired renal function can be seen in infants, children and adolescences (2, 3, 7, 8, 11). The most clinically-significant, potential lethal extrarenal manifestation of ADPKD, ICAs rarely occurs in children. In patients with ADPKD there is a significant degree of phenotypic variability in the rate of progression even among family members, implying factors other than the primary mutation, influence the clinical course of ADPKD. These include modifying genes, epigenetics, dietary and environmental factors.

Diagnosis

As previously noted, the clinical spectrum of pediatric or early-onset ADPKD is especially broad and on rare occasions may be difficult to distinguish ADPKD from ARPKD especially in newborns. However, ADPKD in early childhood generally presents as

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unilateral or bilateral renal cysts in a normal or in mildly enlarged kidneys. Unfortunately, it is still difficult to predict how ADPKD will progress in children with this presentation. The disease may become more clinically symptomatic or the disease may remain asymptomatic well into adulthood (9, 36, 54, 59, 69-72). The differential diagnosis of ADPKD is further discussed in Chapter 1 of this text.

Pathology

The typical appearance of ADPKD in children by US is one or more renal cysts within enlarged kidneys (see Figure 3a). In ADPKD children, kidney cysts form in glomeruli and all tubular segments (see Figures 3b, 3c, 3d, and 3e). The cyst formation is commonly asymmetric and may occasionally present as unilateral (7, 73). In general, the finding of even a single solitary renal cyst in an at-risk pediatric or adolescent patient should prompt further evaluation as simple cysts are extremely rare in children (7). Glomerular cysts may be a component of ADPKD or can occur as a separate, autosomal dominant disease entity. Unlike ARPKD, in which the cystic lesions are ectatic and remain in continuity with the nephron of origin, in ADPKD enlarging cysts “pinch off” or detached from the tubule and do not remain connected to the urinary stream.

Clinical features

As previously noted the clinical spectrum of pediatric or early-onset ADPKD is particularly broad and there is still little evidence to allow accurate prediction of those patients that will rapidly progress. It may present infrequently as severe disease as noted above may be indistinguishable from ARPKD. More frequently, pediatric ADPKD will presents as unilateral or bilateral renal cysts in near normal or mildly enlarged kidneys. Children with this presentation may be symptomatic or may they may remain asymptomatic into adulthood (9, 36, 54, 59, 69-72). Additional renal manifestations of childhood ADPKD include micro and gross hematuria, hypertension, proteinuria, and rarely abdominal, flank or back pain (7, 8). Overt proteinuria is ordinarily a feature of more advanced structural kidney disease and is rare in children with ADPKD (74) but little to no correlation between microalbuminuria and either kidney volume or function in children with ADPKD has been reported (75, 76).

As with ARPKD, hypertension as early as the newborn periods can be a presenting feature in children with ADPKD. Hypertension often precedes biochemical and clinical manifestations of ADPKD and is an important screening measure for at risk children (2, 54, 68, 69, 77). A strong correlation between hypertension and larger kidneys has been observed in multiple cohorts of ADPKD children (74-76, 78). If the affected parent has hypertension, it is more likely to develop at a greater frequency and at an earlier age in their offspring with ADPKD (79).

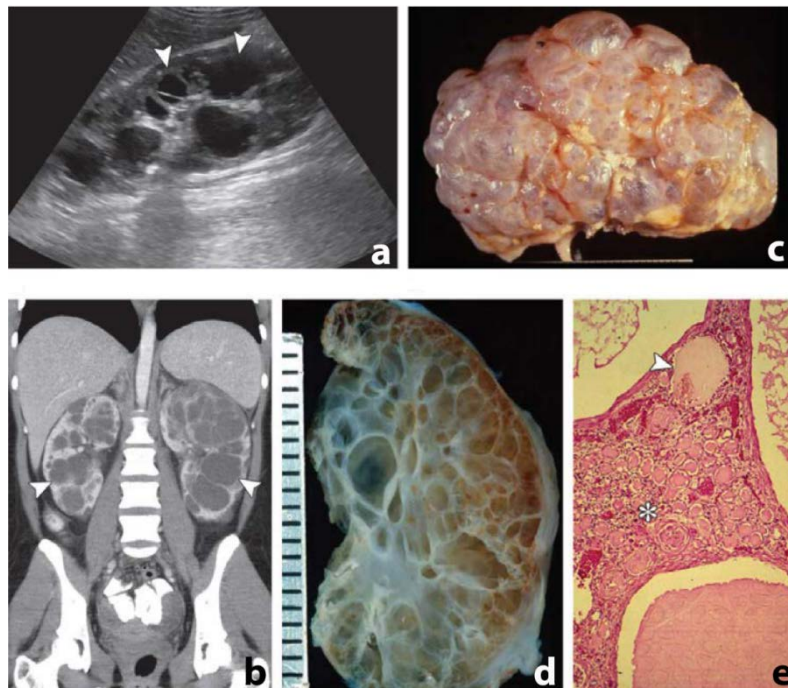


Figure 3. Renal cysts in ADPKD. (a) Longitudinal ultrasound (US) image of the right kidney in a 14-year-old girl reveals multiple round renal cysts (arrowheads). (b) Coronal computed tomography (CT) image in the same patient after administration of intravenous contrast material shows multiple cysts in both kidneys (arrowheads). (c) Photograph of the gross specimen from another patient shows the bosselated surface of the kidney due to many underlying cysts. (d) Photograph of the sectioned gross specimen from another patient shows innumerable round medullary and cortical cysts replacing much of the parenchyma. (Scale is in centimeters.) (e) Photomicrograph (original magnification, $\times 4$; hematoxylin-eosin stain) demonstrates multiple cysts crowding the intervening renal parenchyma with distention of tubules by proteinaceous material (*). Multiple smaller cysts are seen interspersed in the parenchyma as well (arrowhead). Reprinted, with permission, from Chung, et al. *RadioGraphics* 2014; 34:155-178 (43), and the Radiological Society of North America (RSNA).

The use of ambulatory blood pressure monitoring (ABPM) to assess blood pressure (BP) in ADPKD patients has permitted greater precision and revealed abnormalities of BP in ADPKD patients. Nearly 33% of children with ADPKD demonstrate exclusively nocturnal hypertension (80, 81). A significant proportion of normotensive young adults with ADPKD have “prehypertension” by ABPM (68, 82). Blunted “nocturnal dipping” on ABPM has also been reported to be associated with endothelial dysfunction in this population (83). The pathogenesis of hypertension in ADPKD is multifactorial and beyond the scope of this chapter. Excellent reviews are available (68).

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Cardiovascular disease is a major feature of ADPKD, and children with ADPKD demonstrate a substantial correlation between left ventricular mass index (LVMI) and systolic blood pressure (68, 84). Hypertensive and pre-hypertensive (blood pressure in the upper range of normal (75–95th percentile for age, sex, and height) children with ADPKD have a statistically significantly higher LVMI than normotensive ADPKD children (8, 78, 84).

Adult ADPKD patients have an increased incidence of cardiac valvular abnormalities such as mitral valve prolapse, and these abnormalities have also been reported in children with ADPKD (36, 84, 85). In addition to hypertension, other presenting symptoms can include abdominal pain, palpable abdominal masses, gross or microscopic hematuria, UTIs, and abdominal or inguinal hernias. The occurrence of gross hematuria after minor trauma to the side or back should raise the possibility of ADPKD in at risk children. Renal insufficiency in children with ADPKD has been reported but it is generally rare (4, 12, 69, 71). A renal concentrating defect was found in 58% of children with ADPKD in a cohort of children with ADPKD (10, 86). The finding of a renal concentrating defect was shown to correlate with the presence of hypertension by ABPM (87). These findings suggest that impaired renal concentrating ability may be a rough measure of disease severity in children with ADPKD. Renal infections can be a presenting feature in an infant or child with ADPKD (54). Pain from renal stones or a ruptured cyst rupture can also occur in children with ADPKD.

The extrarenal cysts seen in adults with ADPKD (85, 88) occur infrequently in pediatric patients. However, can they provide clinical criteria for a differential diagnosis if found in a child with ADPKD (11). Liver cysts (detected by US) were thought to be a rare finding in children with ADPKD, but a recent MRI study found liver cysts in 55% of adolescents and young adults with ADPKD (89). Liver cysts in children, when present, are not generally associated with pain, infection, and hepatomegaly.

Treatment and complications

Currently treatment options for ADPKD patients is limited to management of renal and extra-renal complications. Asymptomatic children at risk for ADPKD should be closely monitored especially for the development of hypertension and pre-hypertension, hematuria, polyuria, proteinuria or palpable abdominal masses. Any of these findings should prompt further evaluation.

Identification and treatment of hypertension in children is vital in order to slow progression to ESKD in ADPKD. In adults with ADPKD, more intensive blood pressure control (<120/80)

was reported to have a greater impact on LVH reduction than standard control (<140/90) (90). Recent report from the HALT/PKD study failed to demonstrate any benefit gained by adding an angiotensin receptor blocker (ARB) telmisartan to an angiotensin converting enzyme inhibitor (ACEi) lisinopril on ADPKD progression in adult patients with early or moderately-advanced kidney disease. However, a second arm of the HALT/PKD study in younger patients with moderately-preserved renal function, a beneficial effect on total kidney volume (TKV), decreased urinary albumin excretion and LVH was observed when the blood pressure was reduced to a lower target level with an ACEi (91, 92). These data may be particularly applicable to children especially earlier in the disease process. In a randomized double-blind placebo-controlled phase III clinical trial of pravastatin on height-corrected total kidney volume (HtTKV) and LVMI by MRI, pravastatin was effective in slowing the progression of structural kidney disease in older children and young adults with ADPKD (76).

These considerations notwithstanding, well-established international guidelines for staged therapy of hypertension in pediatric patients should be followed.(93). There is little data regarding specific features of UTIs or renal cyst infections in children with ADPKD but the clinical course and certainly the treatment would likely be similar to that for adult ADPKD patients (94, 95).

Flank pain in pediatric ADPKD patients is unusual most likely due to fewer cysts. However, as the disease progresses, particularly in adolescents, flank pain may require intervention. A recent review summarizes the multifactorial pathogenesis and management of chronic pain in ADPKD (96).

Cerebral aneurysms and bleeding occurs in approximately 10% of ADPKD patients and this trait seems to cluster within families (97-99). However, since this rarely occurs before 20 years of age routine screening of pediatric ADPKD patients is not recommended until they reach the age of 20 years old, (100). Screening by magnetic resonance angiography (MRA) may be recommended for patients with symptoms or a positive family history once they reach 20 years of age (97).

Prognosis

The prognosis of early-onset ADPKD presenting in utero or in the neonate was once thought poor. However, recent studies of "very early onset" ADPKD suggest that the prognosis is not as dire as generally presumed. (4, 101, 102).

Given the findings that aggressive treatment of hypertension reduces LVH and the use of pravastatin slows progression of structural kidney disease as well as reduces LVH,

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screening of at risk patients to identify those at risk for rapid progression has become increasingly important (8, 76). New therapies that effectively and safely reduces the number and/or size of renal cysts will most likely provide maximum benefit when started early in the disease course. A recent systematic review of factors that predict progression in ADPKD can be found in (103) .

Pathophysiology of cyst formation in childhood PKD

Delineation of our understanding of molecular mechanisms that drive pathophysiology of cyst formation in PKD is advancing rapidly. However, abnormal cell-signaling networks ultimately produce disease burden. Therefore, therapeutic interventions are largely based on targeting the complex aberrant cellular signaling which defines the cystic cellular phenotype noted below.

Molecular pathogenesis [See chapters 7 to 16, this text]

It is clear that standard Mendelian genetics are inadequate to predict the severity or the rate of progression of the renal disease nor the severity of extrarenal manifestations in childhood PKD. Complex transcriptional events are clearly major disease modifiers in childhood PKD. Recent findings including: the discovery of hypomorphic or incompletely penetrant alleles (104); homozygosity involving either PKD1 or PKD2 (105); compound heterozygosity (23); digenic inheritance or trans-heterozygosity (106); somatic and germline mosaicism (107); genetic modifiers (108) and epigenetic regulators (109, 110) co-inheritance of mutations in either PKD1 or PKD2 with other PKD-causing genes such as HNF1B (36, 111) or the tuberous sclerosis 2 (TSC2) gene (112), all demonstrate that complex inheritance patterns contribute to disease severity and progression in ADPKD and ARPKD.

Cellular pathophysiology of cyst formation in PKD

Cyst development and growth is a complex, multi-factorial process and no single element acts independently. The exact mechanisms that lead to the PKD phenotype is still unclear; however, multiple cellular defects have been identified. Molecular factors that have been shown to influence the extent or severity of cyst formation include: the developmental timing of PKD1 inactivation (113, 114); reduction in functional PC1 dosage (105, 115, 116); cellular and nephron differences in sensitivity to PC1 dosage (115) and the influence of one cyst on neighboring nephrons creating a “snowball effect” leading to cyst development in adjacent tubules (117).

The defining features of a PKD cystic epithelial cell include: a changeover from a mature differentiated, nonproliferative, absorptive cell to a partially dedifferentiated; secretory cell characterized by specific polarization defects; and increased rates of proliferation as well as apoptosis. These changes leading to a proliferating secretory cell is a fundamental and critical change because mathematical modeling of renal cystic epithelia establish proliferation and secretion as necessary and sufficient to account for cyst growth in PKD (118).

Cyst formation in PKD

ADPKD and ARPKD cells share phenotypic abnormalities regardless of when they become clinically-evident. Mutated PKD genes result in abnormal multiprotein complexes whose abnormal function leads to aberrant signaling events resulting in the unique phenotype of the cystic epithelial cell. The precise mechanisms by which these abnormal complexes disrupt normal signaling and cause renal cyst formation are not fully elucidated, significant progress in understanding the cellular events surrounding cyst formation has been made. Key pathogenic features of the unique cystic phenotype have been identified (6, 13, 116, 19-24). These phenotypic abnormalities provide therapeutic targets for the development of PKD therapies, and include:

- abnormalities of in expression of one or more members of the epidermal growth factor (EGF) family of receptors and/or ligands, the (EGFR -axis), leading to an autocrine-paracrine cycle of proliferation through activation of the Ras-Raf-MEK-ERK pathway
- increased cAMP in concert with decreased intracellular calcium levels leads to aberrant intracellular cAMP signaling resulting in activation of the β -Raf /MEK-ERK pathway and stimulates both proliferation and tubular fluid secretion.
- abnormal activity of C-terminal Src kinase or cellular Src (c-Src). A critical molecule that mediates cross-talkbetween the EGFR axis and G-protein-cAMP pathways
- abnormal function of the primary cilia;
- changes in cell-cell, and cell-matrix interactions, and
- alternative activation of renal interstitial macrophages that contribute to the development of progressive fibrosis.

The pathogenic processes listed above all likely contribute at some point to the central characteristic features of renal cyst formation and progressive enlargement, namely: (a) tubular epithelial proliferation; (b) abnormal tubular secretion; and (c) alterations in extracellular matrix, structure, and/or function; and (d) recruitment of macrophages and inflammatory mediators (6, 118, 121, 122, 124-128). Crucial insights into the cellular events

leading to renal cyst formation in PKD have been discovered by orthologous and non-orthologous models of ARPKD and ADPKD. Due to rapid advances in molecular techniques allowing for rapid creation of genetically-manipulated or conditionally targeted models of disease, a comprehensive listing of these models would not be feasible. The interested reader is referred to the following, which review the most significant of these models produced to date (104, 105, 113, 124, 129-131).

The cystic phenotype and targeted future therapy

As discussed in a number of chapters in this text, studies designed to delineate the molecular and cellular biology of ADPKD and ARPKD have defined a unique “cystic phenotype.” This phenotype provides a number of potential targets for the current and future pharmacological therapy (see Figure 4 and Table 1).

Targeted therapeutic strategies can be divided into three categories based upon insights into the pathogenesis of cyst formation in PKD and the aberrant integration of signaling pathways identified to date. These include therapies to: (a) reduce cell proliferation; (b) reduce cAMP levels, and (c) reduce fluid secretion.

Proliferation is an essential process that must be targeted and controlled for any therapy to be effective. There are two main signaling pathways that lead to unchecked proliferation: a) the EGFR family of receptors and ligands; and b) a G-protein regulated axis that leads to increased cAMP and a phenotypic shift in renal epithelia’s response to cAMP. c-Src is a key intermediate that connects both pathways and plays a role in perpetuating the activity of the two pathways. A theoretical sequence of events could follow this step-by-step description: A mutation in PKD1 lead to increased production of amphiregulin, which activates the EGFR axis resulting in: reciprocal phosphorylation (activation) of Src that interacts with the cAMP pathways by altering the response of renal epithelia to cAMP levels, from a normally anti-mitotic to a pro-proliferative response and, in the presence of the terminal tail of PC1, Src dependent activation of STAT3 leads to a persistence of the proliferative signal.. The most promising therapies will most likely target key signaling intermediates that integrate multiple pathways, such as Src, and/or a combination therapy approach where multiple compounds are used to target multiple pathways simultaneously or a single compound that targets multiple pathways such as a multi-kinase inhibitors (MKI) like tesevatinib (132, 133) (“Tesevatinib Ameliorates Progression of ARPKD in Rodent Models” manuscript submitted 9, 2015), currently in clinical trial for ADPKD (ClinicalTrials.gov Identifier NCT01559363).

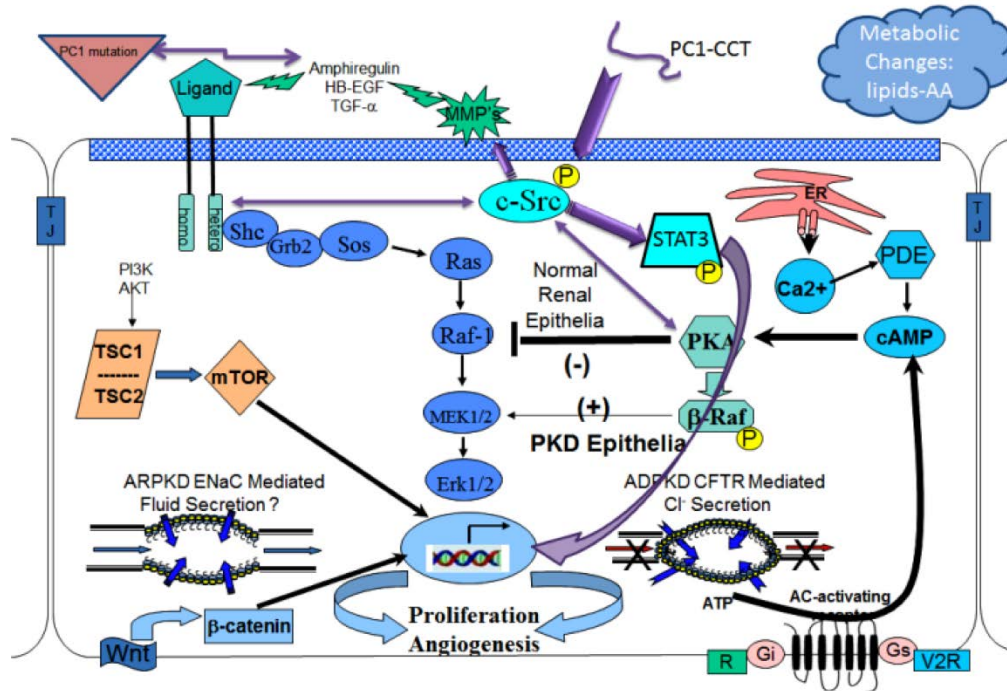


Figure 4. An abridged composite of the abnormally active signal transduction pathways in PKD. This figure represents our approach to the discovery and testing of potential therapeutic interventions. We view proliferation as the critical factor that must be targeted for any therapy to be effective. There are two main conduits that lead to unchecked proliferation: 1) the EGFR axis; and 2) a G-protein axis that leads to increased cAMP and a switch in phenotypic response of renal epithelia to cAMP. Using therapies that inhibit both pathways, a smaller dose will be necessary to achieve maximum cyst reduction and any toxicity associated with the therapy will be minimized. We view c-Src as a critical intermediate that connects both conduits and plays a critical role in integrating the signaling from both conduits. Mutations in PKD1 lead to increased production of amphiregulin, an EGFR ligand which activates the EGFR axis resulting in: reciprocal phosphorylation (activation) of Src that alters the response of renal epithelia to cAMP, from a normally anti-mitotic to a pro-proliferative response. In the presence of the cleaved terminal tail of PC1, Src-dependent activation of STAT3 leads to increased proliferation. Src activation of STAT 3 is amplified by the increased activity of both the EGFR axis and cAMP axis thereby further driving the proliferation of tubular epithelium.

Table 1. The future: potential therapies for childhood PKD

CLASS	ADPKD	ARPKD	EVIDENCE Clinical trials (www.clinicaltrials.gov) Preclinical Studies (PCS) (ref#)
VPV2R antagonist	+/-	+/- (renal only)	multiple CTs ongoing
Src-i	+/?	+/?	CT- Phase II-III
mTOR-i	-/?	-/?	CTs (poor results to date)
Multikinase-i	+/?	+/?	CT for ADPKD in progress
Somastatin and/or analogues	+/?	+/?	CTs for ADPKD in progress
ACEi and ARB	+/? (early disease)	?/?	HALT-PKD (91)
	--/? (late disease)	?/?	HALT-PKD (92)
Statins	?/?	?/?	CT (small cohort) (76)
Triptolide	?/?	?/?	CT (China only) PCS (135)
EGFR axis inhibitors	+/?	+/?	PCS (136)
Angiogenesis-i (+ other therapy)	+/?	+/?	PCS (137)
MMP-i	+/?	+/?	PCS (138)
SMAC Mimetics	+/?	+/?	PCS (139)
HDAC-I	+/?	?/?	PCS (140-142)
Bromodomain Protein-i	+/?	?/?	PCS-(143)
MIF-i	+/?	?/?	PCS-(144)
TNF- α -i	-/?	+/?	PCS (145)
20-HETE-i	+/?	+/?	PCS (146)
ROS-i	+/?	+/?	PCS (147)
CDK inhibitors	+/?	+/?	PCS (148)
HSP90-i	??	??	PCS (149)
Manipulation genetic modifiers	??	??	PCS (150)

The table lists potential therapies based upon pre-clinical trial studies (PCS) or (CT= clinical trials). Updated listing of clinical trials can be found at (www.clinicaltrials.gov).

A number of agents in advanced states of pre-clinical development or Phase 2-3 clinical trials are discussed in Chapter 6. Current listings of ongoing clinical trials for ADPKD and ARPKD can be found at (www.pkdcure.org; and <http://clinicaltrials.gov/>).

Conclusion

The diagnosis of childhood PKD is no longer the terminal diagnosis it once was. For children with ARPKD advances in neonatal critical care and renal replacement therapy have allowed many to survive much longer than what was possible just a few decades ago. Insights into the development and treatment of PH is preventing lethal complications of hepatobiliary disease and provides for a better quality of life. Renal transplantation and dual organ transplantation provides an opportunity for these children to live a near normal life. Pre-implantation genetic diagnosis holds the possibility of eliminating this ARPKD from families who can afford the procedure and who aren't ethically opposed.

For families afflicted with ADPKD, screening of non-symptomatic at-risk offspring, once almost universally discouraged, may change given the evidence that tight control of hypertension can dramatically reduce development of LVH and that pravastatin can dramatically slow structural renal damage from ADPKD. The development of targeted therapies for PKD, and the fact that early intervention should provide the greatest benefit may also increase the number of at-risk offspring being screened.

Despite the extraordinary progress made to date in understanding the molecular and cellular mechanisms of cyst formation in PKD much remains to be done. As our understanding of the molecular mechanisms improves, we must couple this knowledge with new techniques that allow for the rapid creation of animal models that more accurately represent the human disease state. These models will provide increased understanding and new insights into the molecular and cellular mechanisms of PKD that will generate new therapeutic targets as well as provide the basis for the development of even better pre-clinical models of PKD.

Therapies will become increasingly focused on treating PKD in early childhood where they are likely to provide the maximal benefit. The strict control of hypertension will remain an absolute essential component of PKD therapies in the future. Therapies that currently target abnormal signaling pathways will be carefully balanced so that pathway activity is reduced to "normal" or wildtype levels rather than eliminated completely. Modifications to promising compounds will be developed to guide the molecule to the site of need, making these therapies highly specific with very low levels of toxicity (134). This specificity will speed the development of protocols for the ethical treatment of children with PKD where early intervention may allow a lifetime free from the deleterious effects of PKD. Epigenetic and dietary factors that slow or hasten the progression of PKD will be uncovered and adherence or avoidance of such factors may slow the progression of PKD and eliminate the need for pharmacological intervention or renal replacement therapy for some.

As clinical trials to date have shown, targeting a single molecule of pathway has not brought the promising reduction of disease burden as originally hoped for. It is unlikely that any single therapy or compound will be effective especially at all stages of disease. Rational therapies will require knowledge of the extent of disease, the rate of progression, and how this is best assessed. In the near term, therapeutic intervention will likely involve multiple compounds, and the choice of compounds or targets will be stage specific and change as disease progresses.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 3

Treatment and Management of Autosomal Dominant Polycystic Kidney Disease

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the most common life-threatening single-gene disease. It affects up to 15 million people worldwide with 50% risk for end-stage kidney disease, 80% risk for hypertension, 60% risk for painful kidney complications, 20% risk for symptomatic polycystic liver disease and 3% risk for intracerebral aneurysm rupture. For a long time, the treatment and management strategies of this disease have not progressed in comparison with the treatment of other kidney

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diseases. Recently, there have been new therapeutic hopes with identification of specific drugs based on the mechanisms of kidney progression. This chapter reviews the treatment and management of ADPKD progression, and the identification of ADPKD patients with rapidly progressing disease, hypertension, and extrarenal complications.

Key words: ADPKD; Chronic kidney disease; Hypertension; Treatment

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common life-threatening single-gene disease associated with significant morbidity and mortality (1-3). It affects up to 15 million people and represent the 4th most common cause for end-stage kidney disease worldwide (ESKD) (4). ADPKD is a multi-systemic and progressive disorder characterized by cyst formation and enlargement in the kidney and other organs. The natural history of ADPKD was characterized by progressive increase of renal volume and decline of glomerular filtration rate (GFR) (5). Up to 50% of patients with ADPKD require renal replacement therapy by 60 years of age (6). For a long time, the treatment and management strategies of this disease have not been progressed in comparison with the treatment of others kidney diseases. Recently, there have been new therapeutic hopes with identification of specific drugs based on the mechanisms of kidney progression (7). In this chapter, we will summarize the key findings, highlight recent developments, and look ahead to the changes in clinical practice that will likely arise from the adoption of an updated management framework for this major kidney disease.

Management of kidney disease progression in ADPKD

ADPKD progression is highly variable among families and among individuals within families (8), with relative risk of renal decline varying significantly across the population. ADPKD is a significantly heterogeneous disease, making the prediction of progression for a given individual a challenging process. In the absence of reliable and readily available biomarkers and long-term cohort data sets, disease modelling may play a role in defining the wider clinical assessment of at-risk patients and assist in practical decision making (9, 10). Total renal volume is the best predictor of progression of CKD (11).

Vasopressin and the associated cAMP-related signaling pathways have been demonstrated as important contributors for cyst growth in ADPKD, providing rationale for the investigation of vasopressin (V2) receptor antagonism on cystogenesis and proliferation

(12). In 5-year prospective study of Tolvaptan versus placebo, ADPKD patients have been demonstrated a decreased rate of total renal volume growth and a slower decline in kidney function (13). Ensuring that Tolvaptan is used in a safe and effective manner requires multiple considerations, including the careful selection of patients eligible for treatment, based on risk of progression. Patient will need to be supported throughout treatment initiation and long-term management. Japan and the European Medical Agency have approved Tolvaptan for treatment of ADPKD in adults with stage 1 to 3 chronic kidney disease at the outset and evidence of rapidly progressing disease. In the USA, the FDA requested additional data to further evaluate the efficacy and safety of this drug. Identifying ADPKD patients with evidence of rapidly progressing disease requires a consensus to define which scores to use. In addition, results of randomized studies in later stages of ADPKD are not encouraging, which suggests that use of Tolvaptan is not recommended without the additional evidence from large clinical trials.

Hypertension management in ADPKD

Hypertension is a common and serious complication of ADPKD, often occurring in 50% - 70% of ADPKD patients early in the disease, before appearance of renal dysfunction (14, 15). The median age at diagnosis of hypertension in ADPKD is 32 years for males and 34 years for females (16) and occurs at an earlier age in comparison with general population (17). Furthermore, hypertension occurs in 30% of children with ADPKD (18-21). Early and effective treatment of hypertension is very important to decrease the morbidity and mortality of ADPKD patients.

The pathogenesis of hypertension in ADPKD is complex and dependent on many factors that influence each other. Activation of the renin-angiotensin-aldosterone system (RAAS), vascular dysfunction related to ciliopathy, activation of efferent sympathetic nerves, renal handling of sodium and others factors have all been found to be involved in the development of hypertension in ADPKD (22-24). Activation of the RAAS seems to have a major role in the pathogenesis of hypertension in ADPKD patients (25).

In patients with renal disease, the goal is a blood pressure of less than 130/80 mm Hg. In patients with ADPKD we have to adopt this blood pressure target. Hypertension is the most important modifiable risk factor in ADPKD and better blood pressure control allows slowing down the progression of kidney disease (26).

Based on pathogenic data of hypertension in ADPKD patients, the best treatment of this disease is RAAS inhibitors with angiotensin-converting enzyme (ACE) inhibitors or

angiotensin II receptor blockers (ARBs). These agents remain the most recommended drugs to treat hypertension in patients with ADPKD, although studies of the RAAS have not convincingly demonstrated that it plays an important role in the pathogenesis of ADPKD. At the moment, there are not sufficient studies that examined the effect of hypertension control on kidney disease progression and occurrence of cardiovascular events (27). In early stages of ADPKD (28), the dual inhibition of RAAS by combination of ACE inhibitor and ARB did not significantly decrease the rate of total kidney volume growth. Rigorous blood-pressure control was associated with a slower rate of total kidney volume growth in comparison with standard blood pressure control, a greater decline in the left ventricular mass index, and greater reduction in urinary albumin excretion. However, no effect on kidney function was observed. In later stages of ADPKD (29), monotherapy blockade of RAAS with an ACE inhibitor was associated with blood pressure control in ADPKD patients with stage 3 chronic kidney disease. The addition of an ARB did not affect the kidney function progression.

Current published data confirm that patients with ADPKD in the United States (30, 31), Denmark (32) and Great Britain (33) are having a better prognosis. There has been earlier diagnosis, better control of blood pressure, more use of RAAS inhibitors, better preservation of renal function, later onset of ESKD, and better survival. Therefore, early control of hypertension is very important in patients with ADPKD to slow down kidney disease progression and prevent occurrence of cardiovascular events (30-33). The improved survival no doubt involves factors in addition to the better control of blood pressure and preservation of renal function, and this issue therefore needs further study.

Extrarenal complications management

Co-morbidity from extrarenal manifestations is largely confined to adult patients. Hepatic cysts develop later than renal cysts and are rarely found in children. Their prevalence reaches 80% after 60 years of age (34). Most patients remain asymptomatic, with preserved liver function. Females tend to have more cysts and multiple pregnancies and use of estrogen increases cyst size and number. Persistent and severe pain may require cyst decompression. Infection of hepatic cysts is rare and requires antibiotics and sometimes drainage. Medical management using somatostatin analogs has led to significant reduction in liver volume with continued use (35). Epithelial cysts in other organs are infrequently seen. These include pancreas, ovaries, spleen, thyroid, endometrium, seminal vesicle and the epididymis. Along with the progressive cyst development in the kidney and other organs, patients with ADPKD are at increased risk for a variety of vascular abnormalities (36, 37). Intracranial aneurysms have been found in 8% of patients, compared to 1.2% in the

general population, and appear to be clustered in families (1, 38). Rupture of aneurysms is the most serious complication in ADPKD and may account for 7% to 13% of deaths in ADPKD. Management of unruptured aneurysms should be discussed with a multidisciplinary team at an expert center. Aneurysms of the aorta and cardiac valve abnormalities have also been reported (39). It is uncertain whether vascular complications result directly from the genetic defect or merely as a consequence of hypertension and renal failure in these patients. In young children extrarenal manifestations have only rarely been noted.

Urinary tract infections may lead to cyst infection, renal abscesses and sepsis and are considered to be risk factors in progression of renal disease (40). These infections may be difficult to treat. Adequate treatment with antibiotics that can penetrate cyst walls is critical (41). Macroscopic and microscopic hematuria may result from a rupturing cyst and is usually self-limiting. Reduced physical activity may be recommended rarely in cases of protracted bleeding.

Pain from ADPKD, sometimes associated with perinephric hemorrhage, can be treated with analgesics. When the pain persists for more than a few days one must consider the possibility of renal infection, stones or tumor. Pain may also be associated with enlargement of cysts. In such cases some relief may be obtained from percutaneous aspiration or surgical reduction of cysts (42). Renal denervation has been used successfully and could be performed concurrently with cyst decortications (42, 43).

ADPKD progresses to ESKD in approximately 50% of patients at 60 years of age (6). Progression appears to be faster in those who have the PKD1 as opposed to the PKD2 genotype (40). Hypertension and kidney infections are considered most important modifiable risk factors for the development of renal failure and should therefore be adequately treated. A slowdown of the progression to ESKD by early treatment of normotensive patients with ACE inhibitors may be hypothesized, but has not yet been established. Reduction in dietary protein intake has shown disappointing results on slowing progression of renal disease (44).

Earlier onset of chronic kidney disease has been related to a younger age at diagnosis, larger kidneys, episodes of hematuria, proteinuria and multiple pregnancies (40). Understanding predictors for rapid progression of this disease has become increasingly important with the emergence of potential new treatments. Several risk factors influencing kidney disease progression in ADPKD have been identified in the current era. Early emergent markers of ADPKD renal disease progression, specifically, total kidney volume, glomerular hyperfiltration, renal blood flow, uric acid, and urinary molecular markers

Table 1. Management of ADPKD patients (13, 15, 46, 47)

Manifestation	Recommendation
Assess for the presence of risk factors for rapidly progressing disease	<ul style="list-style-type: none"> • PKD-1 gene mutation; • Male gender; • Young age at diagnosis; • Presence of hypertension, • Hematuria • Proteinuria; • Young age at onset of hypertension • Increased total kidney volume
Reduce hypertension risk	<ul style="list-style-type: none"> • Lifestyle changes -- Smoking cessation -- Dietary salt restriction -- Moderate alcohol consumption -- Maintain BMI between 18.5 and 24.9 kg/m² through diet and exercise -- Avoid caffeinated drinks • BP: assess and maintain BP <130 / 80 mmHg with RAAS inhibitors
Slow kidney disease progression	<ul style="list-style-type: none"> • Lifestyle changes, • Tolvaptan approved for ADPKD adults patients with CKD stage 1 to 3 and evidence of rapidly progressing disease
Assess and manage other renal complications <ul style="list-style-type: none"> • Chronic kidney disease • Renal pain • Kidney stones 	<ul style="list-style-type: none"> • Monitor eGFR and refer to nephrologist • Symptom review • Maintain adequate fluid intake (3 liters per day) for primary prevention
<ul style="list-style-type: none"> • Hematuria • Urinary tract and cyst infection • Large symptomatic cyst 	<ul style="list-style-type: none"> • Reduced physical activity • Antibiotics , fluoroquinolones • Consider avoiding oral contraceptive pill and hormone replacement therapy in women with severe polycystic liver disease • Aspiration and sclerotherapy, • Cyst fenestration, resection
Assess and manage other systemic complications <ul style="list-style-type: none"> • Intracranial aneurysms 	<ul style="list-style-type: none"> • Consider screening for intracranial cerebral aneurysm in ADPKD patients with high risk • Clipping or endovascular procedure

ADPKD (autosomal dominant polycystic kidney disease); BP (blood pressure); BMI (body mass index); CKD (chronic kidney disease); ESKD (end-stage kidney disease); eGFR (glomerular filtration rate); RAAS (renin-angiotensin-aldosterone system); TVK (Total kidney volume).

have been implicated (9, 10). ADPKD patients with ESRD can be dialyzed and receive renal transplants equally well as patients with most other renal disorders. Patients at-risk for the development of cerebral aneurysms, including those with a positive family history, should be screened by cerebral computed tomography and/or magnetic resonance imaging (45). Asymptomatic at-risk children in ADPKD families are usually followed-up annually for the development of hypertension, hematuria and urinary tract infections. Hypertension and urinary tract infections need prompt and adequate treatment because these may enhance the progression of renal lesions.

Lifestyle modifications, consultations and long-term monitoring in ADPKD

Patients with ADPKD should avoid violent sports. All ADPKD female of reproductive potential should receive counseling on potential aggravation of polycystic liver disease with exogenous estrogen or progesterone exposure. Non-hypertensive ADPKD patients with normal kidney function should undergo blood and urinary testing and ultrasonography of the kidneys every year. ADPKD patients with high blood pressure, chronic kidney disease or cardiovascular complications require more frequent monitoring, based on the severity of hypertension and stage of chronic kidney disease.

ADPKD is the most common hereditary renal disease in the adult. Strategies of treatment and management should be individualized for each ADPKD patient. The objective of this chapter is to provide an update approach and current state of knowledge related to the evaluation, management and treatment of ADPKD (Table 1). Recent recommendations for ADPKD have been published to help with improving disease management and treatment (46, 47).

Conclusion

Improvements in screening and diagnosis of ADPKD have allowed earlier diagnosis of disease, later onset of ESRD and better survival. However, the main and most effective therapy remains control of hypertension. Therefore, early and effective treatment of hypertension is very important to decrease the morbidity and mortality of ADPKD patients. Tolvaptan, a V2 receptor antagonist, was demonstrated to be effective in slowing deterioration of renal function and renal volume growth. Currently, we have new tools and early markers to monitor and detect complications earlier such as total kidney volume. Nephrologists should regularly followed-up ADPKD patients to screen earlier the other complications related to ADPKD for early management.

Conflict of interest

The author declares that he has no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 4

Diagnosis and Treatment Modalities of Symptomatic Polycystic Kidney Disease

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Abstract

Polycystic kidney disease (PKD) can cause end stage kidney disease with an autosomal dominant inheritance pattern. Besides renal replacement therapy or renal transplantation, there are no other curative therapies. Renal insufficiency, severe pain due to hemorrhagic expansion of the cysts, or infections are the most common clinical presentations. Diagnosis of infected cysts can be quite challenging. In recent years, 18FDG-PET/CT has shown to be the most sensitive and accurate modality for the diagnosis of infected cysts. The majority of these infections respond to systemic antibiotic therapy, but in some cases, percutaneous drainage is indicated. In some cases, the volume of the native polycystic kidneys is so

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extensive that native nephrectomy is necessary to create enough space in the iliac fossa to allow the placement of a renal graft. Tolvaptan, a selective arginine vasopressin V2 receptor antagonist, can be used to reduce the speed of disease progression in selected patients. Trans-arterial embolization has shown to be safe and effective to downsize very large native kidneys and it can be beneficial for patients who are at high risk for surgery or who decline surgery. The aim of our chapter is to present the current literature on the best diagnostic tests for patients with suspected infected or hemorrhagic cysts, and the best treatment modalities for patients with symptomatic polycystic kidneys prior or after renal transplantation.

Key words: Diagnosis; Genetic Mutations; Therapy; Transarterial Embolization; Vasopressin Receptor Antagonists

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) represents the fourth leading cause of end-stage kidney disease (ESKD) in the United States where 33 patients per million initiate dialysis due to progression of their disease every year (1). Mutations of two major genes cause ADPKD by stimulating renal tubule cells to proliferate at increased rates (2). Molecular mechanism and/or defective development and function of the primary cilia are involved in causing cysts to form in the kidneys and in the liver. In the late stages, cysts virtually replace the renal parenchyma (**Figure 1**). Mutated polycystins are expressed in kidney, liver, pancreas, seminal vesicles, brain and endothelium; thus, ADPKD should be considered a systemic disorder (3, 4). Vascular expression of the mutation can be life threatening when it leads to the formation of intracranial and large-vessels aneurysms and cardiac valvular disease (4, 5).

Genetic mutations and molecular abnormalities

PKD can be classified into ADPKD and autosomal recessive PKD (ARPKD) (6). ARPKD is an abnormality of renal tubular development affecting the collecting ducts while ADPKD is an abnormality of the homeostasis of the renal tubules (4). ADPKD is the most common abnormality with an incidence of 1 in 400-1000 individuals and accounts for 5% of patients with ESKD (7) while ARPKD has an incidence of 1 in 20,000-40,000 individuals and tends to manifest early in life (7). ADPKD is due to mutations of Polycystin 1 (PC1) and 2 (PC2), transmembrane glycoproteins found on renal tubular epithelial cells. PC1 and PC2 are the normal proteins that inhibit cell proliferation and mutations of their respective genes are responsible for the development of PKD. Mutations of PKD1 (Chromosome 16) represents 85% of cases while mutations of PKD2 (Chromosome 4) represent the remaining 15%. ARPKD, a rare pediatric form of polycystic kidney disease, is due to mutations of PKHD1 (Chromosome 6), encoding for fibrocystin/polyductin (8). The presence of two completely inactivated PKHD1 alleles results in a more severe clinical outcome associated with perinatal mortality (4) (Table 1).

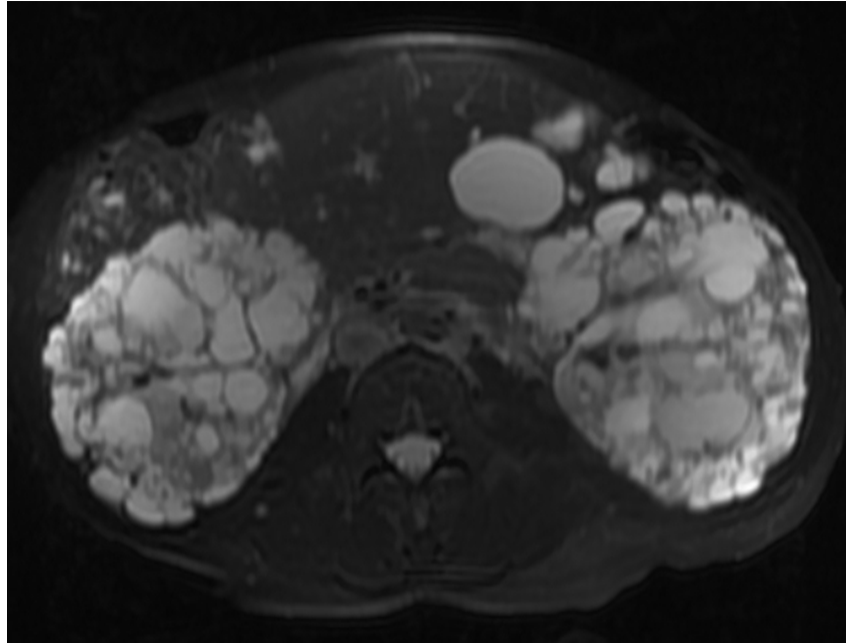


Figure 1. T2 phase MRI of the abdomen and pelvis of a patient affected by autosomal dominant polycystic kidney disease (ADPKD). Both kidneys are affected by multiple cysts of different size. Normal renal parenchyma can be found in the space between the renal cysts. The pressure generated by the fluid that fills the lumen of the cysts progressively compresses normal renal tissue and, over time, 40-50% of patients affected by ADPKD can progress to end stage kidney disease (4). Contrary to autosomal recessive polycystic kidney disease (ARPKD), the renal cysts in ADPKD develop in any part of the nephron and involve the medulla as often as the cortex. Because ADPKD is a progressive disease, most patients are born with normal kidney and a normal ultrasound examination cannot exclude ADPKD until after the age of 35 (5), especially for patients with PKD2 mutations which are associated with later disease onset.

Mutations of PKD1 are associated with more renal cysts and faster progression to renal failure in comparison to mutations of PKD2. Mutations in PKD1 and PKD2 on one allele are not sufficient to cause ADPKD as a second mutation on another allele is necessary to stimulate monoclonal differentiation of tubular cells and development of renal cysts (9). However, despite extensive research efforts, the molecular basis of cyst formation and enlargement remains not completely understood (6).

Table 1. Genetic, clinical and radiological imaging characteristics of autosomal recessive and autosomal dominant polycystic kidney disease

Condition	Genetics	Clinical and Pathologic Features	Imaging Features
ARPKD	PKHD1 (6p12): polyductin-fibrocystin	Renal: oliguria, dilated collecting ducts Pulmonary: hypoplasia Liver: portal hypertension due to congenital hepatic fibrosis, biliary duct dilatation	Echogenic kidneys with tubular cysts, mostly medullary. Hepatic periportal edema and fibrosis, intra and extra-hepatic biliary dilatation
ADPKD	PKD1 (16p13,3): polycystin-1, PKD2 (4q22_ polycystin-2	Renal: cortical and medullary cysts Liver: hepatic cysts Other organs: cysts of other organs (e.g. pancreas), vascular abnormalities (aneurysms)	Initially normal-sized kidneys with a few round cysts. Hepatic and other abdominal organ cysts in adults

PKD1= Polycystic kidney disease 1; PKD2=polycystic kidney disease 2; ADPKD=autosomal-dominant polycystic kidney disease; ARPKD=autosomal recessive polycystic kidney disease.

In 40% of patients affected by PKD, the condition can be diagnosed *in utero* by ultrasound within the 13th-20th week of gestation (7). Contrary to ADPKD where patients are expected to survive for several decades, infants affected by ARPKD often die shortly after birth due to respiratory failure from pulmonary complications due to hypoplasia as the results of oligohydramnios for the insufficient fetal urine output or due to pneumothorax (1, 4). ARPKD is also associated with biliary dysgenesis where the intrahepatic bile ducts increase in number and dilate with subsequent periportal fibrosis and portal hypertension (4, 10) and children who survive to adolescence tend to develop liver and biliary dysfunction later in life that might require liver transplantation (4, 11). Overall, 20-year outcomes after diagnosis of ARPKD depend largely on the age at presentation with 36% survival rate when diagnosis is made at less than 1 year of age, 80% with presentation at 1-20 years of age, and 88% with presentation older than 20 years of age (12).

Diagnosis of PKD

ADPKD is characterized by the development of bilateral tubular ectasias of the collecting ducts (renal cysts), kidney pain, hypertension and progressive loss of renal function. Although ADPKD cannot be differentiated from ARPKD only on the basis of ultrasonographic findings, the presence of renal cysts in a fetus, favours ADPKD since ultrasound in neonates with ARPKD usually reveal massively-enlarged, smooth, hyperechogenic kidneys with lack of normal corticomedullary differentiation but rarely well-formed cysts (4, 13). The single most reliable means of differentiating between these two hereditary cystic diseases is to perform renal ultrasound in the parents. The diagnosis of ARPKD is favoured if neither parent has the characteristic findings of ADPKD. When the diagnosis of PKD is uncertain, molecular testing can be obtained by

gene sequencing of PKHD1 or by linkage analysis (14). Direct molecular genetic tests have an accuracy of only 80% since it cannot detect all mutations responsible for ARPKD (15,16). Despite this limitation, one of the strengths of molecular test is that it is the only one able to provide predictive information about ARPKD before the clinical signs and symptoms develop.

One of the greatest challenges of ADPKD is to make the diagnosis in patients younger than 30 years and without a positive family history since the formation of renal cysts is age dependent and the disease may be caused by new mutations in up to 15% of cases (17). In fact, unlike patients with ARPKD, most patients with ADPKD are born with normal kidneys and present with renal insufficiency or hypertension in adulthood, when much of their kidneys have been replaced by cysts (4). Currently, molecular testing is recommended for individuals under the age of 30 at risk of being affected by PKD and with less than 3 renal cysts on ultrasound (18, 19).

Ultrasonography is the most cost-effective radiological modality for the diagnosis of PKD (1) while MRI should be used when ultrasound is inconclusive (20). The following diagnostic criteria are used for patients suspected with PKD according to their age:

- For patients aged 15-39 years the presence of 3 or more unilateral or bilateral cysts has a sensitivity of 0.7 and specificity of 1, positive predictive value of 1 and negative predictive value of 0.7.
- For patients aged 40-59 the presence of 2 or more unilateral or bilateral cysts has a sensitivity of 1, specificity of 0.9, positive predictive value of 0.9 and negative predictive value of 1.
- For patients aged 60 years or older the presence of 4 or more cysts in each kidney has a sensitivity of 1 and specificity of 1.

Natural history of ADPKD

The rate of disease progression is quite variable and the expected increase in total kidney volume can range from 1-10% per year (21). Approximately 50% of patients with ADPKD will develop ESKD in their fourth to sixth decades of life. However, many factors modify the course and severity of ADPKD and its natural history still remains poorly understood (6). Even among members of the same family with the same germline mutation, there is marked variability in disease severity (6). Interval assessment of the radiological changes in kidney volume is the best method of monitoring patients with ADPKD. The factors that are associated with worse renal outcome include PKD1 mutation in contrast to PKD2, male gender, African origin, sickle cell disease, earlier age of presentation, presence of hypertension, episodes of gross hematuria, recurrent urinary tract infections and low HDL(2, 5, 9, 22-24).

Table 2. Manifestations of Autosomal Dominant Polycystic Kidney Disease

Renal Manifestations	Extra-renal Manifestations
Renal Cysts	Gastrointestinal
Renal Adenomas	Hepatic cysts
Renal cell carcinoma	Pancreatic cysts
Hypertension	Diverticulosis
Hematuria / Hemorrhage	Congenital hepatic fibrosis (rare)
Acute and chronic pain	Cholangiocarcinoma (rare)
Urinary tract infections	Cardiovascular
Nephrolithiasis	Valvular abnormalities
Nephromegaly	Intracranial aneurysm
Renal failure	Thoracic and abdominal aneurysm
	Other Systems
	Ovarian cysts
	Arachnoid cysts (rare)
	Pineal cysts (rare)
	Splenic cysts (rare)

Symptoms of ADPKD

Unilateral or bilateral lumbar pain is a common symptom of ADPKD that can lower patients' quality of life (QOL). Rarely, patients require narcotics to control their symptoms. Other common manifestations associated with ADPKD are abdominal distension and early satiety due to the compression of surrounding gastrointestinal organs, hematuria, urinary tract infections, infections of the renal cysts and nephrolithiasis (Table 2).

Therapy of ADPKD and management of symptomatic polycystic kidneys

Dialysis or renal transplantation, are the only treatment options for patients with ADPKD that has progressed to ESKD. Recent studies have shown that selective arginine vasopressin V2 receptor antagonists may be beneficial for patients with early renal dysfunction. Among them, Tolvaptan has been the most promising to delay progression of the disease in patients with a modest degree of renal insufficiency. For the rest of the patients with ADPKD, treatment options are limited to palliation of their symptoms. Anti-inflammatory medications and narcotics are used to control severe abdominal or back pain,

broad spectrum antibiotics, specifically floxacins, for the treatment of infections of the urinary system and of the renal cysts, and embolization of renal arteries for the treatment of macroscopic hematuria. In recent years, arterial embolization has been accepted as an effective technique to decrease the volume of large and symptomatic polycystic kidneys as an alternative to the more traditional surgical approach (25).

Treatment with Tolvaptan

Tolvaptan is orally active, has a half-life of about 12 hours and it is approved for the treatment of hyponatremia. Tolvaptan has received approval for the treatment of ADPKD in Canada, Great Britain, Europe and Japan. Patients taking Tolvaptan must drink volumes of water reaching 4 to 5 liters per day; consequently, its use is associated with aquaretic side effects (polyuria, nocturia, and rarely, hypernatremia). Hepatotoxicity, manifested by elevated liver enzymes has been observed, but is reversible upon withdrawal of the drug. Elevated plasma uric acid concentrations and gout may also be encountered. Therefore, the benefits of Tolvaptan must be carefully weighed against the associated risks for each individual patient.

Recent evidence has highlighted the beneficial effect of Tolvaptan on delaying the progression of ADPKD. The TEMPO 2:4 trial examined long-term (3 years) safety, tolerability and efficacy of Tolvaptan in a multicenter open-label study (26, 27). Overall 96% of patients taking a daily dose of 60 mg tolerated the treatment well and had an annual total kidney volume change of $1.7 \pm 3.5\%$ compared to $5.8 \pm 4.3\%$ for the control group ($p < 0.01$). These findings were confirmed by a subsequent randomized phase III multicenter double blind placebo controlled trial TEMPO 3:4 (28). The benefits of Tolvaptan appeared enhanced in patients older than 35 years, with hypertension or total kidney volume of 1500 ml or higher at baseline. Limited data of the effect of Tolvaptan are available for patients with more advanced ADPKD. Expert opinion and the results of these randomized studies indicate that any intervention in later stages of ADPKD is likely to be futile in slowing the progression of the disease and therefore the routine use of Tolvaptan is not recommended without the additional evidence from large clinical trials.

Trans-arterial embolization

ADPKD can produce severe, often intractable abdominal pain and flank pain, which is attributed to progressive cyst dilation and displacement of renal parenchyma. This process is responsible for nerve irritation within the renal parenchyma, the intrarenal collecting system and renal capsule. In ADPKD with ESKD, nephrectomy is one of the treatment options for pain relief. However, nephrectomy of patients with very large renal volumes can be technically challenging and associated with risks of bleeding, incisional hernias, superficial and deep infections and intestinal perforation (29). In addition to the morbidity of the surgical intervention, patients might be exposed to blood products with the

subsequent formation of allo-antibodies that decrease their chances of human leukocyte antigens (HLA) matching for future renal transplants. Some studies have shown that surgical decompression of cysts is safe and effective for patients with intractable pain when a dominant cyst is suspected to be the main cause of symptoms (30). If a few cysts are present, another suitable option is percutaneous aspiration with chemical ablation of the cysts. However, when the cysts are numerous, this approach is ineffective since it is technically very difficult to drain and ablate all the potential cysts that might be responsible for the symptoms.

In recent years, there have been an increasing number of observational studies reporting good outcomes with trans-arterial embolization (TAE) of the affected kidneys. TAE has been extensively used for treating renal tumors, intrarenal aneurysms and hematuria due to trauma or renal biopsies and most of the interventional radiologists are proficient with the procedure that is usually performed using Seldinger's technique with intravenous sedation in combination with local anesthesia at the arterial puncture site. TAE produces a reduction in renal volume because the volume expansion of cysts is the results of an active and complex cellular process requiring energy and involving the development of an extensive capillary network in the cyst wall secondary to an increase in the level of vascular endothelial growth factor (VEGF) (2, 31). After accessing the femoral artery, renal artery occlusion is usually obtained by infusing microspheres of polyvinyl alcohol under fluoroscopic guidance initially into intrarenal branches using small particles (diameter, 100-300 μm) and then with larger particles (diameter, 300-500 μm) whenever necessary. Using this technique, Cornelis et al. (25) were able to treat 25 patients waiting for a renal transplantation and reduce the volume of their native kidneys so that the patients did not have to undergo pre-transplantation nephrectomies to allow implantation of the grafts in the iliac fossa. In their experience, the mean reduction in volume was 42% at 3 months and 54% at 6 months and TAE was successful in 85% of cases. One patient required additional cyst sclerosis to reach the objective and the absence of sufficient volume reduction in the remaining cases was due to an excessive basal renal volume, missed accessory artery and or renal artery revascularization.

Nephrectomy

The rate of nephrectomy in patients with ADPKD has steadily declined over the past decade due to significant improvements in conservative therapy. Initially, bilateral native nephrectomy for complicated ADPKD was associated with significant complications and 3% mortality (32). Although more modern series including minimally invasive approaches show much lower morbidity and mortality rates (33, 34), there is consensus that most of the ADPKD patients do not require native nephrectomy to facilitate kidney transplantation (35). Therefore, modern management of patients with ADPKD tends to avoid pre-transplant bilateral native nephrectomy to preserve endogenous erythropoietin production, and to maintain better quality of life by sustained urine production. Nevertheless, for

patients who are symptomatic or who have native kidneys that occupy the iliac fossa preventing renal transplantation due to lack of space, native kidneys nephrectomy is necessary. Yet, the timing of nephrectomy (before or during transplantation), and whether it should be performed bilaterally or unilaterally or by open or laparoscopic approach, have been a subject of much debate (22, 35, 36). Since Elashry et al. (37) first described a laparoscopic approach for removing a polycystic kidney, multiple studies have shown the feasibility of this approach with some describing laparoscopic simultaneous bilateral native nephrectomies (38, 39). Benefits of the laparoscopic approach include shorter hospital stays, decreased morbidity and quicker recovery (40). Additionally, when performed as a combined procedure with a simultaneous renal transplant, the laparoscopic approach offers similar morbidity to renal transplantation alone, without graft compromise and with the convenience of a single operation (33). However, some ADPKD kidneys can be severely enlarged, essentially filling the abdominal cavity and often crossing the midline. Laparoscopy in such cases can be daunting, and some have advocated the open approach for these particularly challenging cases where the renal volume for each kidney is more than 2,5 – 3 liters (41).

Native nephrectomy can be performed prior, during or following transplantation. An alternative method is the so called “sandwich technique” where the most severely affected native kidney is removed prior or during transplantation, and the other native kidney is removed subsequently to avoid possible complications due to infections, haemorrhage or pain caused by the native PKD. The advantage of performing unilateral native nephrectomy before transplantation is that the patient can be scheduled for the operation in an elective fashion, the removal of the enlarged kidney facilitates future renal transplantation and patients might experience significant improvement in their quality of life due to decreased abdominal distension and pain. The disadvantage of this approach is that patients require at least two operations: one for the native nephrectomy and another one for the renal transplantation. In addition, some patients will need a third operation to excise the contralateral native kidney if it becomes symptomatic. Neef et al. reported that contralateral native nephrectomy was necessary in 26% of patients who underwent renal transplantation and simultaneous unilateral native nephrectomy due to various reasons including suspected malignancy, infections or bleeding (42). This observation was supported by recent data indicating that more than 40% of patients with ADPKD with a native kidney left in situ after transplantation require nephrectomy due to complications if followed long enough (43). Another important consideration is that some patients might require blood transfusions during or after native nephrectomy and their risk of developing allo-antibodies increases with consequent decrease of their chance of matching with a potential donor. On the other hand, Neef et al. (42) reported that complications rates and graft losses were highest among patients without pre-transplant nephrectomy in comparison to the control group due to ongoing hematuria and recurrent infections after kidney engrafting. Several other studies have also shown better graft function and post-operative survival in patients treated with unilateral or bilateral nephrectomy before

transplantation compared to patients without this additional procedure (36, 44) since the most common cause for adverse outcomes impacting both patient and graft survival were septic complications directly related to the retained polycystic kidneys (36, 44-46).

One of the benefits of performing simultaneous unilateral native nephrectomy and renal transplantation is that patients undergo one operation that can be performed using an extended Gibson incision, it reduces overall hospital stay and it is cosmetically attractive as patients will have only one surgical scar. Unilateral nephrectomy accomplished via an extension of the Gibson incision, increases the operative time of only 20-45 minutes (42) versus bilateral simultaneous nephrectomy, which is usually performed via a transperitoneal access and lengthens the operation time by 180 minutes (47). Drawbacks of this method is that the transplant operation is significantly longer and technically more challenging especially when performed during cadaveric transplantation after regular hours and the postoperative management of these patients is more complex as perioperative fluid shift is more extensive.

The option of performing simultaneous bilateral native nephrectomies and renal transplantation appears attractive as it is the most expeditious way of dealing with the potential risk of post-transplant complications due to the presence of polycystic kidneys. Tyson et al. (48) used the Nationwide Inpatient Sample (NIS) registry to assess the outcome of patients with PKD who underwent simultaneous renal transplantation and bilateral native nephrectomy and patients who underwent bilateral native nephrectomy alone. These authors found that among 2,368 patients who satisfied the inclusion criteria, 271 (11.4%) underwent simultaneous renal transplantation and developed higher rates of intraoperative haemorrhage, blood transfusion and urological complications in comparison to the control group. However, in-hospital mortality was lower in patients undergoing simultaneous bilateral nephrectomy and renal transplantation. Several other groups noted acceptable results and argued that simultaneous nephrectomy and transplantation are the preferred strategy because it avoids the negative effects of being anephric as well as the toll of 2 operations (34, 49).

Infections

The estimated incidence of infections of renal cysts in patients with ADPKD is 1 episode per 100 patients per year (50). Known predisposing factors include advanced age, female gender and instrumentation of the urinary tract (51) as the presumed mechanism of the infection is the retrograde migration of bacteria through the ureters while hematogenous seeding is less likely (51).

The diagnosis of cystic infections is usually based on clinical grounds when patients develop systemic symptoms such as fever, weight loss and malaise often in combination

with abdominal or back pain (52). The diagnosis can be quite straightforward when symptoms are combined with elevation of laboratory markers of inflammation such as erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and fibrinogen but there are no established cut off values for these parameters. On the other hand, to prove that a patient is indeed affected by infection of a renal cyst is quite difficult as the gold standard requires the analysis of the content of the responsible cyst. This is often not feasible nor recommended especially when there is no clear identification of which cyst is infected.

Common pathogens include *E. coli* (74% of positive cultures) (50) and other enteric flora and cyst infections accounts for 10-15% of all causes of hospitalizations of ADPKD patients (50, 51). These infections can have serious consequences (51), and patients who are waiting for a renal transplant should be put on hold until their infection is resolved since bacteremia and sepsis can result in graft loss or perioperative death. Similarly, patients who are immunosuppressed after renal transplantation can develop serious infections in the native polycystic kidneys cysts that can be responsible for systemic complications due to their attenuated immune response (51). Sallee et al. (50) have proposed clinical criteria for the diagnosis of infected cysts in patients with ADPKD. These criteria are: 1) presence of neutrophils and bacteria in the fluid aspirate from the suspected cyst; or alternatively, 2) the concurrent manifestation of fever (temperature $>38^{\circ}\text{C}$ for > 3 days), abdominal tenderness in the area of the polycystic kidney, increased serum C-reactive protein levels (CRP, $>5\text{ mg/dL}$) and the absence of computed tomography (CT) findings suggestive for recent intracystic bleeding (intracystic density < 25 Hounsfield units).

Although these criteria are important to initiate and direct the duration of systemic antibiotic therapy (53), they are not helpful for the identification of which cysts are infected. Currently, there is no diagnostic imaging gold standard (54) and conventional cross sectional imaging modalities, including ultrasounds (US), CT and magnetic resonance scans (MRI) are only valuable in discriminating between non-complicated and complicated cyst and are unable to discriminate between bleeding, infection or early neoplasia (23).

In addition, the diagnostic accuracy of these tests is further limited by the fact that contrast agents cannot be used in the presence of renal dysfunction in patients with ADPKD (2). Recently, there has been some enthusiasm around the use of scintigraphy with indium- or gallium-labelled leukocytes as some investigators have reported promising results in localizing infected cysts (55). However, one of the limitations of this technique is the fact that it is not universally available, it is quite costly and provides a relatively poor spatial discrimination when patients have severe anatomical distortion of their native kidneys (56, 57).

Positron Emission Tomography (PET) using 18-Fluorodeoxyglucose (18FDG) has shown some promising role for the accurate diagnosis and localization of suspected infected renal cysts (51, 58). One of the advantages of 18FDG is that it is not nephrotoxic and can be

successfully used in patients with ESKD (59). In addition, when associated with CT scanning, 18FDG-PET/CT has good spatial discrimination, which may allow the guiding of percutaneous procedures or the study of the adjacent tissues (60). Lantinga et al. (61) evaluated the diagnostic criteria in renal and hepatic cyst infection and found that 18FDG-PET/CT identified 100% of definitive and 93% of probable cyst infection cases. Another group (59) also reported that 18FDG-PET/CT yielded positive results in 87% of cases of infected cysts. Jouret et al. (59) reported that in their experience 18FDG-PET was able to identify distinct non-cystic infectious conditions such as ischemic colitis, diverticulitis, retroperitoneal abscesses, prostatitis, pyelonephritis, and infected abdominal aorta aneurysm and that 18FDG-PET changed the management of 26% of patients who were initially diagnosed with suspected infected renal cysts. The excellent sensitivity of 18FDG-PET and the spatial discrimination obtained by combining 18FDG-PET with CT reported by Lantiga et al. (61) and more recently by Jouret et al. (59) should be explored further by other well-designed studies to determine the exact diagnostic sensitivity and specificity of 18FDG-PET/CT across a wide spectrum of disease presentations. In fact, the diagnostic performance of 18FDG-PET has not been fully evaluated for intracystic bleeding that is the main differential diagnosis of cyst infection in ADPKD patients and accumulation of 18FDG has been described in the setting of hematomas outside the renal parenchyma (62). Therefore, the specificity of 18FDG-PET/CT for renal cyst infections remains unknown at this point. Another important drawback of this diagnostic modality is that although preliminary data indicate that 18FDG-PET/CT is a promising tool for the diagnosis and follow-up of infected renal cysts, the low availability and its high costs that are similar to scintigraphy with labeled white blood cells will limit its wider use.

The main treatment for suspected infected renal cysts is systemic antibiotic therapy for 3 to 6 weeks (50). The selection of the type of antibiotic is usually empirical as the results of blood and urine cultures lag behind the clinical presentation. Despite this limitation, in a large cohort of patients treated in the United Kingdom, the clinical efficacy of the initial antibiotic therapy was observed in 71% of infections (50). Fluoroquinolones and third-generation cephalosporins are the most common choice as they cover the majority of the common Gram-negative bacteria responsible for the infections and they have a relative good passive diffusion profile that allow them to accumulate in the lumen of renal cysts (50). Fluoroquinolones are usually favored because of their superior lipophilic properties in comparison to β -lactamines, mainly penicillins and cephalosporins that are known to have poor penetration in larger renal cysts. In selected patients with good renal function or on haemodialysis, aminoglycosides can be used as monotherapy or in combination with other antibiotics when other antibiotics fail (22, 53).

The majority of patients respond to antibiotic therapy without the need for any other intervention. However, for a small group, percutaneous drainage of suspected large (>5 cm) infected cysts may be beneficial as antibiotics often do not have the ability to reach the concentration necessary to sterilize the cystic fluid (50, 63).

Conclusion and perspective

Approximately 40-50% of patients affected by PKD are symptomatic and clinicians should be familiar with all the possible modalities that are currently available to care for these challenging patients. Tolvaptan, a selective arginine vasopressin V2 receptor antagonist, has been shown to reduce the speed of disease progression in selected patients. Diagnosis of infected cysts can be quite challenging and 18FDG-PET/CT has shown to be the most sensitive and accurate imaging modality. The majority of these infections respond to systemic antibiotic therapy, but in some cases, percutaneous drainage is necessary. The identification of the infectious agent by blood and urine cultures is essential in tailoring the type and duration of the antibiotic therapy. Surgical excision of native polycystic kidneys is necessary when patients are symptomatic and fail other modalities. Surgical therapy is also indicated to remove large volume native kidney that occupy the iliac fossa of patients in need of renal transplantation. In recent years, TAE has shown to be safe and effective to downsize very large native kidneys and it can be beneficial for patients who are at high risk or who decline surgery.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 5

Blood Pressure Control for Polycystic Kidney Disease

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent genetic kidney disease and affects 6 to 12 million patients worldwide. The disease is characterized by the progressive development of innumerable renal cysts that gradually replace normal kidney tissue, leading ultimately to the loss of renal function starting from the 5th decade of life. Most patients with ADPKD develop arterial hypertension. High blood pressure

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develops early in the course of the disease and is caused by the activation of the renin-angiotensin-aldosterone system (RAAS) and other significant pathogenic mechanisms. Hypertension is a major contributing factor for the increased cardiovascular morbidity and mortality in patients with ADPKD. Optimal treatment of hypertension is essential to improve the prognosis of the cystic disease and the associated cardiovascular diseases. Target blood pressures and choice of antihypertensive drugs for patients with ADPKD have not been firmly defined in guidelines, but the recently published results from the HALT-PKD studies suggest that a blood pressure goal of <130/80 mm Hg should be targeted, preferably with inhibitors of the RAAS.

Key Words: Hypertension; Polycystic kidney disease; Renin-angiotensin-aldosterone-system

Introduction

The autosomal dominant form of polycystic kidney disease (ADPKD) is the most common monogenic kidney disease in humans and is the cause of end-stage kidney disease (ESKD) in 7-10% of dialysis patients (1). Mutations in the PKD1 gene (85% of cases; clinically severe form) and PKD2 (15% of cases; clinically more slowly progressive form) are primarily responsible for the disease (2). ADPKD is usually manifested between 25 and 45 years of age. The most common primary manifestations include 1) arterial hypertension at early age; 2) abdominal or flank pain, often in the context of cyst hemorrhage or cyst infection; 3) hematuria, mostly microscopic but sometimes macroscopic as a manifestation of cyst bleeding; and 4) moderate polyuria, manifesting as nocturia. The progression of ADPKD is characterized by a large inter-individual variability. Patients with PKD1 mutation reach ESKD in their mid-fifties, and those with PKD2 mutations in their mid-seventies (3).

The disease is characterized by increasing formation and expansion of fluid-filled cysts in the parenchyma of both kidneys. During the course of the disease, there is progressive enlargement of the cysts which is due to aberrant proliferation of the cyst epithelial cells and the secretion of cyst fluid. Thereby, the surrounding kidney tissue is compressed and injured, leading ultimately to a reduction of the glomerular filtration rate (GFR). At the start of dialysis, the kidneys are massively enlarged due to the growth of the cysts, and the normal renal parenchyma has been replaced by atrophic tubules and fibrotic areas.

In addition to the above mentioned renal characteristics, patients with ADPKD develop several well characterized extrarenal manifestations which include the formation of cysts in the liver and pancreas, colonic diverticula, abdominal hernias, mitral valve prolapse (rarely mitral insufficiency), aortic valve anomalies and the dreaded intracranial aneurysms. The

latter may lead to rupture and potentially fatal subarachnoid hemorrhage. Fortunately this complication is rare, however aneurysms occur more frequently in certain families (4).

Arterial hypertension in ADPKD

General aspects

Arterial hypertension occurs in patients with ADPKD relatively early in the disease course, usually much earlier than in the general population (5, 6). The median age at diagnosis of hypertension is 32 years for men and 34 years for women (7). In case of a PKD1 mutation, treatment for hypertension is required about 5 years earlier than in case of a PKD2 mutation, and 50-70% of patients will develop hypertension before renal function is limited (8). The extent of hypertension correlates with the volume and the growth rate of the renal cysts (9). The development of left ventricular hypertrophy (LVH) is tightly linked to arterial hypertension and is associated with an increased cardiovascular risk in these patients. Thus, hypertension is a modifiable risk factor for cardiovascular diseases in ADPKD patients and should be treated in order to reduce the burden of cardiovascular complications. In addition, there is more and more evidence that hypertension contributes to disease progression in ADPKD. Early diagnosis and optimal treatment are therefore of great importance, first of all to prevent cardiovascular complications in these patients, and then also to retard cyst growth (10, 11).

Pathophysiology of hypertension in ADPKD

Hypertension occurs in patients with ADPKD when the excretory renal function is still normal and thus cannot exclusively be explained by altered salt excretion or other non-specific mechanisms of renal hypertension. Rather, a number of specific mechanisms seem to be responsible for the development of hypertension in ADPKD (Figure 1) (12, 13). The RAAS plays a predominant role, since cyst formation leads to compression of vessels and creates local ischemia which leads to the activation of the intrarenal RAAS. This has been demonstrated in numerous experimental and clinical studies (12). In addition, the sympathetic nervous system is activated in ADPKD and contributes to the pathogenesis of hypertension (14). Furthermore, due to the renal concentrating defect and the consecutive tendency to polyuria, a latent stimulation of vascular vasopressin V1 receptors occurs, leading to vasoconstriction and a consecutive increase in blood pressure. Finally, polycystin 1 and 2 are expressed by endothelial cells and vascular smooth muscle cells where they contribute to the regulation of the vascular tone through endothelin, nitric oxide (NO) and the homeostasis of intracellular calcium (8, 12).

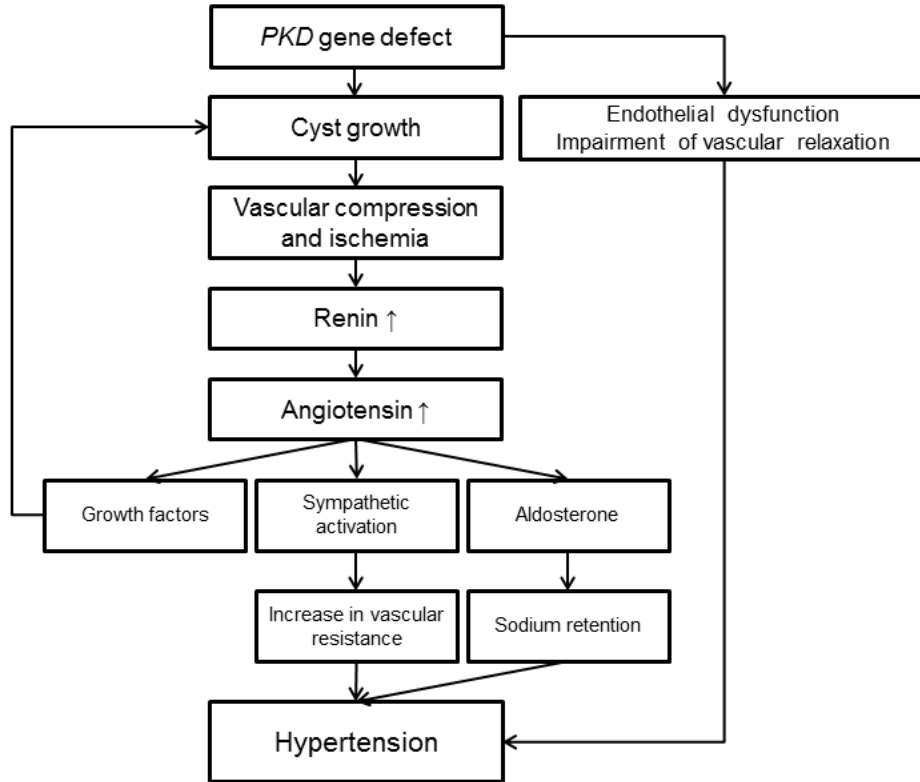


Figure 1. Pathogenesis of hypertension in autosomal dominant polycystic kidney disease (ADPKD). The major pathogenic mechanisms for hypertension are depicted, highlighting the important role of the RAAS.

Treatment of hypertension in ADPKD

General aspects

Early and timely initiation of effective and long-term treatment is essential in the management of hypertension in patients with ADPKD (15). The development of hypertension can in general be equated with a progression of cyst growth. It has been recommended that blood pressure target values with drug treatment should aim to values of <130/80 mm Hg in adults, and below the 75th percentile in children (16). As in other patients with hypertension, the goals of antihypertensive therapy consist in the reduction of

extrarenal complications (LVH, arteriosclerosis) and hypertensive renal damage (nephroangiosclerosis, glomerulosclerosis). In addition there is evidence that intrarenal RAAS activation and hypertension may favor cyst growth. Thus, animal studies have shown that Angiotensin Converting Enzyme Inhibitors (ACEI) could arrest the cyst growth by antagonizing the mitogenic effect of the RAAS (17, 18). When treating hypertension in ADPKD the following three questions need to be addressed: (1) which drug classes (or non-drug therapies) are preferable; (2) what is the optimal blood pressure target and at which blood pressure values should drug therapy be initiated; (3) do ACEI or Angiotensin Receptor Blockers (ARB) have protective effects which are independent of blood pressure control?

Therapeutic options for the treatment of hypertension in ADPKD

In patients with ADPKD the general principles of non-drug treatment of hypertension apply, including, limiting the intake of salt and caffeine, regular exercise and smoking cessation. In general, high blood pressure in patients with ADPKD is relatively easy to treat, because drug resistance is quite rare. Often, a single medication is sufficient to control hypertension, particularly when combined with a thiazide diuretic. Due to pathophysiological considerations, RAAS inhibitors (ACEI and ARB) appear to be well suited as first choice therapy, particularly in light of the recently published results of the large HALT-PKD studies (see below). There is no study with a sufficiently large patient number that has demonstrated the superiority of RAAS inhibitors against other blood pressure regimens, but there are many small studies which provide indirect evidence that ACEI/ARB are the preferred classes of antihypertensive compounds. The following small-scale studies are noteworthy:

- In a non-randomized study in hypertensive ADPKD patients (n=33), GFR declined faster with a diuretic than with an ACEI. The annual decrease in creatinine clearance was 5.3 ml/min/1.73 m² in the diuretic group and 2.7 ml/min/1.73 m² in the ACEI group (P<0.05) (19).
- In a prospective randomized study of 24 patients, the effects of the calcium channel blocker amlodipine and the ACEI enalapril on blood pressure, proteinuria and GFR over 5 years was examined. At comparable blood pressure control only enalapril reduced the proteinuria, but both drugs showed a similar decline in GFR (20).
- In another small prospective study, 49 patients were randomized and treated with amlodipine or the ACEI candesartan for 3 years. At comparable blood pressure control, it was observed that, with amlodipine, 24% of the patients showed a doubling of serum creatinine compared to only 4.2% with candesartan (21).

- A small and non-conclusive 2-year study in 26 patients comparing the effect of calcium channel blockers with ACEI and found no difference in blood pressure control or serum creatinine (22).
- A retrospective study of 32 patients also documented a greater loss of GFR with calcium channel blockers than with RAAS inhibition of ACEI or ARB (23).
- A study in 61 normotensive and 28 hypertensive ADPKD patients compared the ACEI enalapril with the beta-blocker atenolol or placebo and found no difference in GFR loss (24).
- A study of 85 children with ADPKD showed that enalapril could stabilize renal function and left ventricular mass index but that the kidney volume continued to increase (25).
- Finally, a meta-analysis of 11 randomized clinical trials was performed which studied 1860 patients with non-diabetic nephropathies, including 142 patients with ADPKD. Compared with other blood pressure medications, ACEI were more effective in reducing proteinuria, particularly in patients with advanced ADPKD, and especially in patients with larger proteinuria. However, the influence of ACEI on the progression towards renal failure was not conclusive. Of note, the ADPKD study population seemed to be unusual, since a significantly higher proteinuria was found in these patients at baseline (26).

In summary, up to the year 2014 there was no clear evidence for superior efficacy and safety of RAAS blockers over other antihypertensive drugs, particularly with respect to clinically relevant endpoints such as GFR decline and total kidney volume (TKV) growth. The above mentioned studies were too small and of too short duration to provide conclusive results. Nevertheless, these smaller studies have provided good evidence that inhibition of the RAAS with ACEI or ARB allows effective and safe treatment of hypertension in ADPKD and that they should be preferred over calcium channel blockers or diuretics alone.

Blood pressure targets in ADPKD

Disease-specific blood pressure targets have not yet been conclusively defined for ADPKD. A subgroup analysis of the Modification of Diet in Renal Disease (MDRD) study was performed in 200 patients with ADPKD with a GFR of 25 to 55 ml/min/1.73 m², showing no difference between normal (mean arterial pressure [MAP] target ≤ 113 mm Hg over 60

Hypertension in ADPKD

years and ≤ 107 below 60 years) and strict (MAP target ≤ 92 mm Hg over 60 years and ≤ 88 below 60 years) blood pressure control. However, in patients with a GFR of 13 to 24 ml/min/1.73 m² there was a slightly more rapid GFR decline in the group with strict blood pressure control (27). Another randomized controlled trial with 75 ADPKD patients found no difference in renal function with a strict ($<120/80$ mm Hg) compared to a normal (135-140/85-90 mm Hg) blood pressure regimen using enalapril or amlodipine over 7 years. However, a significant positive effect was found on LVH (28).

HALT-PKD results

The question of blood pressure target values and whether the progression of renal disease (cyst growth and GFR loss) can be inhibited by a dual RAAS blockade were the focus of the HALT-PKD study program. It has been so far the largest and methodologically well-conducted study on the treatment of hypertension in ADPKD (29, 30).

The HALT-PKD study program was carried out from 2006 to 2014. It examined the effect of RAAS blockade and strict control of blood pressure on the progression of renal disease in adults with ADPKD (Figure 2). This study examined the effect of RAAS blockade with the ACEI lisinopril alone or in combination with the ARB telmisartan on the progression of the disease in patients with preserved GFR (Study A; GFR >60 ml/min/1.73 m²; n = 558) and in patients with advanced renal disease (Study B; GFR 25-60 ml/min/1.73 m²; n = 486). Study A had a 2x2 factorial design: patients were randomly assigned to one of two blood pressure goals [standard BP (120/70 to 130/80 mm Hg) versus low BP (95/60 to 110/75 mm Hg)] and to either monotherapy with an ACEI or dual RAS blockade ([lisinopril + placebo] versus [lisinopril + telmisartan]). In Study B, [lisinopril + placebo] versus [lisinopril + telmisartan] were compared at the same target blood pressure goal (110-130/70-80 mm Hg). In Study A, the primary endpoint was defined as the TKV, while in study B, the combined primary end point evaluated the improvement of the GFR decline, occurrence of end-stage kidney failure and death.

In study A, patients with the lower BP target had a significant reduction of the kidney volume growth (5.6 vs 6.6%, $P = 0.006$), thus meeting the primary endpoint of the study, although the effect was small (Figure 3). However, there was no difference in the level of renal function (GFR annual loss -2.9 vs -3.0 ml/min/1.73 m²) (Table 1). A significant reduction of left ventricular mass index (LVMI) and albuminuria was observed in the treatment group with the lower BP goal, but it resulted in frequent orthostatic complaints such as dizziness. When [lisinopril + placebo] was compared with [lisinopril + telmisartan], there was no difference in the growth of kidney volume (Figure 3), GFR loss, albuminuria and LVMI.

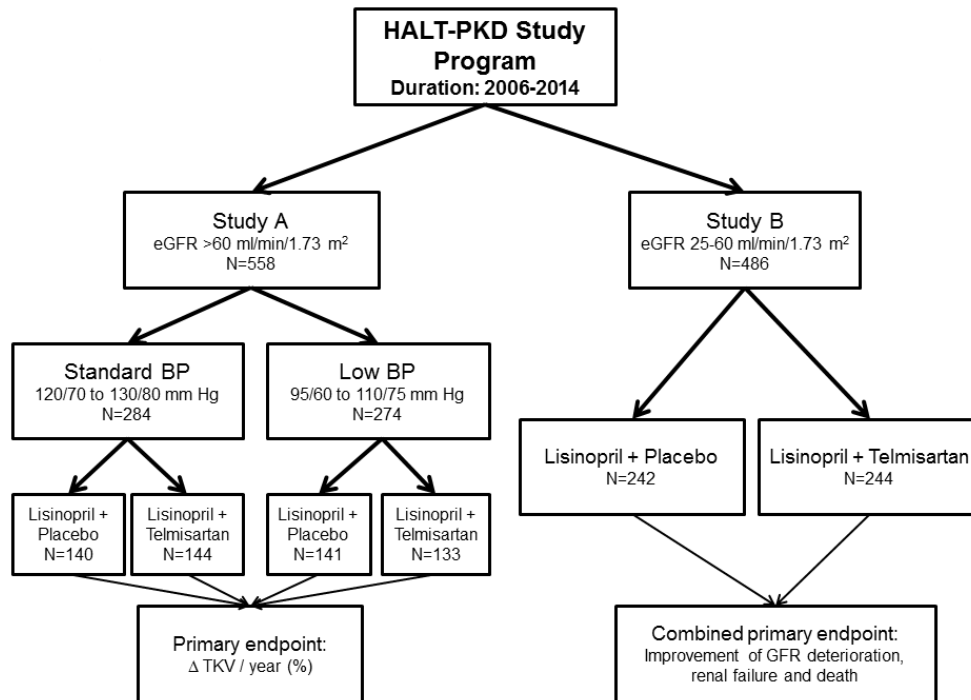


Figure 2. Schematic representation of the HALT-PKD study program and the eGFR criteria for inclusion in study A (preserved GFR) and B (reduced GFR). Study A has a 2x2 factorial design (standard vs. lower BP, and comparing lisinopril/placebo vs. lisinopril/telmisartan).

In Study B, patients in both treatment groups [lisinopril + placebo] versus [lisinopril + telmisartan] had more advanced cystic disease than in study A (i.e. older age, lower GFR). There was no difference in the combined primary endpoint (improvement of GFR decline, reaching ESKD or death), and no difference in GFR loss and albuminuria between the treatment groups. Under dual therapy with lisinopril and telmisartan, the risk of hyperkalemia and acute renal failure was not increased.

In summary, the HALT-PKD study results suggest that lowering BP more intensely in earlier stages of ADPKD has a favorable effect on the course of the disease, but at the cost of increased hypotensive side effects. Dual RAAS blockade was comparable to monotherapy with an ACEI, even in patients with advanced disease. Of note, the complication rate was not increased with dual therapy, unlike other studies with diabetic patients where a higher risk of hyperkalemia and acute renal failure has been noticed (31).

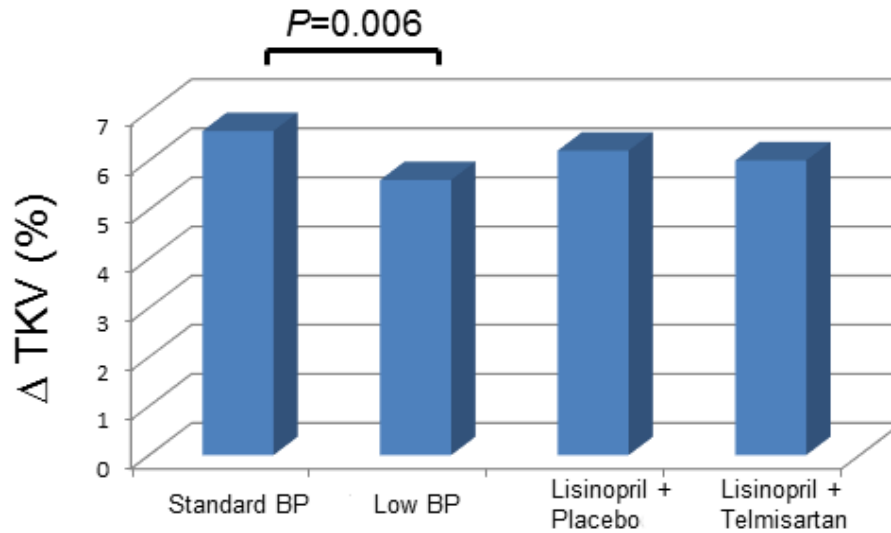


Figure 3. Annual change in total kidney volume (Δ TKV) in percent in the HALT-PKD Study A. The lower BP was associated with decreased growth (5.6 vs 6.6%, relative difference 14.2%, $P = 0.006$), whereas the addition of telmisartan to lisinopril did not change TKV growth (6.0 vs 6.2%, $P = 0.52$).

Finally, it must be noted that in both studies (A and B) the dose of the ACEI in the dual RAAS blockade group was lower than in the ACEI monotherapy group. Furthermore, the blood pressure was similar in both treatment groups and there was no difference in the suppression of urine aldosterone. Hence, it can be concluded that the suppression of the RAAS in both treatment groups was comparable and the combination of ACEI and ARB was not more effective than monotherapy with an ACEI at a higher dose. Unfortunately, there was no control arm without inhibitors of the RAAS in the HALT-PKD studies, so ultimately it is still unclear whether the inhibition of the RAAS to control blood pressure is superior to other drug regimens (beta-blockers and/or diuretics). Nevertheless, a lower BP appears to significantly inhibit renal cyst growth, but it remains to be seen whether this strategy can delay the occurrence of ESKD.

Table 1. Study results of the HALT-PKD studies (from Schrier (29) and Torres (30))

	Study A	Study B
Number of participants	558	486
Age category	15-49 years	18-64 years
GFR	>60 ml/min/1.73 m ²	25-60 ml/min/1.73 m ²
Study arms	Standard (120-130/70-80 mm Hg) vs. low (95-110/60-75 mm Hg) blood pressure Lisinopril + Placebo (L/P) vs. Lisinopril + Telmisartan (L/T)	Lisinopril + Placebo vs. Lisinopril + Telmisartan
Primary Endpoint	Change in cyst growth in low blood pressure group: Δ TKV 5.6% vs. 6.6% Similar cyst growth in L/P and L/T: Δ TKV 6.2% vs. 6.0%	50% reduction of eGFR, ESKD or death: HR 1.08, CI 0.82 - 1.42
Secondary Endpoint	Significant reduction of LVMI and albuminuria at a low BP, no difference in eGFR loss No differences between L/P and L/T with respect to LVMI, albuminuria and eGFR loss	No difference in eGFR and albuminuria loss
Side Effects	Dizziness and light-headedness more common in low BP group No differences between L/P and L/T	No difference in hyperkalemia and acute renal failure

eGFR, estimated Glomerular Filtration Rate; ESKD, End-Stage Kidney Disease; L/P, Lisinopril + Placebo treatment arm; L/T, Lisinopril + Telmisartan treatment arm. Δ TKV, yearly change in Total Kidney Volume.

Conclusions for clinical practice

Patients with ADPKD develop hypertension early in the disease process. Hypertension contributes to disease progression and increased cardiovascular risk. Generally, hypertension is easy to treat and resistant forms are very rare. Because there is marked intrarenal activation of the RAAS, treatment with ACEI or ARB, optionally in combination with a thiazide diuretic, is recommended as the preferred drug regimen. The target blood pressure should be below 130/80 mm Hg. Based on new data from the HALT-PKD studies, a greater blood pressure reduction can be recommended, provided that patients tolerate it and do not develop hypotensive side effects.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 6

Clinical Trials in Autosomal Dominant Polycystic Kidney Disease

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Abstract

Autosomal dominant polycystic kidney disease is the most common life-threatening genetic disease, affecting 1/400 to 1/1000 live births. It represents the 4th leading cause of end-stage kidney disease and accounts for 13% of kidney transplants in the United States. It is characterized by irreversible cyst growth leading to progressive parenchymal damage. Despite promising results, there is currently no Food and Drug Administration approved therapy to cure or slow the progression of the disease. The growing understanding of the

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pathophysiological mechanisms leading to cyst formation and growth has led to the development of several therapeutic agents, some with very promising results. In this chapter, we will review the past and ongoing clinical trials that explored these specific targets, focusing mainly on drugs targeting the cyclic adenosine 3',5'-monophosphate pathway (vasopressin V2 receptor antagonists and somatostatin analogs), mammalian target of rapamycin (mTOR) inhibitors and renin-angiotensin blockade. We will discuss novel therapeutic targets currently being explored in pilot clinical studies, including high dose niacinamide, tyrosine kinase inhibitors and others. Given its slowly progressive nature and lack of early sensitive biomarkers, clinical trials are limited by the need for long follow-up periods to assess the beneficial effect on kidney function of any therapeutic intervention. There is a growing need of new biomarkers of PKD progression to help accelerate the progress of clinical trials in this field. We will finally explore the current accepted and candidate biomarkers in PKD and discuss current challenges to the development of clinical trials in PKD.

Key words: Clinical trial; Drug therapy; Glomerular filtration rate; Kidney volume

Introduction

The genes for autosomal dominant polycystic kidney disease (ADPKD), PKD1 and PKD2 encoding polycystin-1 (PC1) and polycystin-2 (PC2), respectively, were first identified 20 years ago (1-3). In the intervening years a vast amount of information has emerged regarding the cellular mechanisms and signaling pathways that are dysregulated in PKD, and numerous potential targets and candidate drugs have been proposed (4), but as yet there is no drug approved in the United States for treating the disease. Thus, PKD is "therapy-ripe" and represents a very exciting area for clinical trials. The first 8 interventional trials in PKD have been published in the past five years (Table 1) and we are poised for a deluge of novel drugs and other therapeutic candidates that will need to be tested.

Challenges in drug development and the design of clinical trials in ADPKD

A number of challenges are faced by investigators attempting to bring therapies to clinical trials and ultimately obtain regulatory approval.

Preclinical models may not predict clinical efficacy

The pathogenesis of PKD is complex and still poorly understood. It is fairly well accepted that renal tubule epithelial cell proliferation is increased, and that there is

abnormal secretion of fluid into the lumen. Thus, assays of PKD cell proliferation (5) and of cyst growth from PKD cells in 3D culture (6) have been used to test drugs. However, existing PKD cell lines and primary cells all have their limitations and so it is unclear if in vitro assays truly predict clinical drug efficacy. Rodent PKD models are widely used for preclinical testing but they do not completely mimic the human disease (7). Most require homozygous gene deletion from birth or early embryonic life, and then exhibit an accelerated disease progression with numerous cysts developing contemporaneously. By contrast PKD patients are heterozygous and have a slowly progressive disease with cysts of varying ages emerging over several decades of life (8). Moreover, in nonorthologous models that do more closely mimic the human disease (e.g. pcy mouse, or Han:SPRD-cy rat) it is unclear whether the underlying disease mechanism is the same as that for ADPKD. Finally, the rodent kidney is several orders of magnitude smaller and is simpler in structure than the human kidney, so the mechanical effects of cysts on normal kidney tissue may not be faithfully modeled. Some of these limitations will hopefully be overcome with the development of hypomorphic (9) or delayed-onset disease models (10), and large animal models (11).

Difficulty in assessing biological efficacy

In early phase trials, a critical component is determining whether the drug, at the dose and route given, has the intended pharmacodynamic effect on its target in the cystic kidney, for proof of biological efficacy. If the drug has a measurable physiological effect (for example, tolvaptan causing a reduction in urine osmolality) this can be used, but such a convenient readout is unlikely to be available for most drugs. Another possibility is to employ a biomarker that is informative of the drug effect. For technical and ethical reasons cystic kidneys are not generally biopsied. Thus the only practical way to access information about intrarenal biochemical events is to sample soluble factors, cells or exosomes that are excreted in the urine. However, the urine also contains small molecules and peptides filtered from the circulation, as well as cells and probably exosomes shed from the lower urinary tract, diluting the signal of interest. Many intracellular signaling molecules that might be drug targets are not found in urine at all. Finally, cyst fluid, which contains factors secreted from cyst epithelium is usually sealed within large non-communicating cysts and not in continuity with the tubular fluid of functioning nephrons, leaving only the younger and smaller communicating cysts to deliver relevant molecules into the urine.

The use of mammalian target of rapamycin (mTOR) inhibitors for ADPKD illustrates this challenge well. While mTOR inhibitors at high doses were shown to be effective at treating PKD in mice (12, 13), their use in humans, at the usual doses used for immunosuppression and that were known to be well tolerated, showed equivocal or no clinical efficacy in

retarding progression of ADPKD (14, 15) (see detailed discussion below). However, in these studies there was no determination of whether the mTOR pathway in the epithelium of kidney cysts or precystic tubules was effectively inhibited, presumably because there are no urinary biomarkers of this pathway. A fortuitous occurrence in a patient in France provided evidence to suggest that the usual clinical doses of mTOR inhibitors are insufficient to achieve biological efficacy within the PKD kidney (16). This patient was inadvertently transplanted with an ADPKD kidney, given sirolimus for post-transplant immunosuppression and then underwent a routine kidney biopsy at 1-year post-transplant. In peripheral blood mononuclear cells from the patient, phosphorylation of p70 S6 kinase by mTOR was effectively inhibited by sirolimus, confirming that there was a systemic drug effect. However, the kidney biopsy from the same patient showed persistently high levels of both phospho-S6 ribosomal protein and phospho-4E-BP1, indicating absence of biological efficacy of sirolimus within the kidney.

Long natural history of disease

In ADPKD, cysts likely begin forming before birth (17) and grow exponentially throughout the life of those kidneys (18), a duration that averages about six decades (19). During this time, cysts progressively compress and injure neighboring structures, including tubules and vasculature, and incite inflammation and eventually fibrosis. However, the glomerular filtration rate (GFR) remains well-preserved for several decades, likely due to compensatory hyperfiltration of the remaining functional nephron units. Thus, clinical trials are likely to show the greatest therapeutic benefit in early adulthood (or perhaps even in childhood), when most of the damage from cyst growth is being inflicted and is reversible (20). However, at this stage in the disease it is difficult to demonstrate any improvement in the course of the GFR and virtually impossible to assess the rate of progression to end-stage kidney disease (ESKD). This highlights one of the limitations of using improvement in GFR as a measure of drug clinical efficacy (others are discussed in the next two subsections). By the time the GFR begins to decline, there is already extensive kidney fibrosis, there has presumably been so much nephron dropout that the remaining nephrons can no longer compensate adequately, and the kidneys are set on a course of rapid and likely irreversible decline that usually leads to ESKD within a few years. A trial of therapy at this late stage is unlikely to alter the natural history of the disease.

Age-related decline in GFR

GFR declines slowly with age even in normal individuals, with an average slope of approximately $-0.8 \text{ mL/min/1.73 m}^2$ (21, 22). Thus, the slope of GFR in ADPKD patients

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represents the additive effect of two processes: cyst growth-induced kidney injury, which is the target of our therapy, and age-related GFR decline, which presumably would not be ameliorated by PKD therapy. This limits the magnitude of the improvement in GFR slope that we could reasonably expect to see in a clinical trial.

Acute effects of drugs on GFR

A number of drugs have acute hemodynamic effects on GFR that differ from, and mask their long-term effect, on kidney function (23, 24). For example, drugs that block the renin-angiotensin system, such as angiotensin-converting enzyme inhibitors (ACEI) and angiotensin II receptor AT1 blockers (ARB) cause vasodilation of the glomerular efferent arterioles and acutely reduce GFR. However this same effect ameliorates glomerular hypertension and contributes to the long-term effect of these drugs to reduce the slope of decline in GFR (25). In a study in which the outcome is the slope of GFR measured from the start of the trial, before drug treatment is initiated, to the end of the trial while on-drug, the short-term detrimental effect may partially or totally mask the long-term beneficial effect. A similar effect is seen simply with marked lowering of the blood pressure (BP) and probably accounts for the lack of beneficial effect of a low BP target as compared to a standard BP target on the overall slope of the GFR in the HALT A trial (see detailed discussion below) (26).

This phenomenon can be addressed by modifying the study design. In the TEMPO 3:4 study (see detailed discussion below), it was anticipated that the vasopressin receptor-2 antagonist tolvaptan would have a similar hemodynamic effect. The study was therefore designed so that the slope of reciprocal creatinine (a secondary outcome that was used as a surrogate for GFR) was determined “on-drug”, starting after the drug had been titrated to its target dose, and ending with the last encounter in which the patient was still taking the drug (27).

Lack of sensitive early clinical biomarkers acceptable to the FDA as endpoint

Given the aforementioned limitations of using GFR or ESKD as endpoints in clinical trials of ADPKD, much effort has focused on identification of alternative clinical biomarkers that are closely associated with the rate of disease progression and predictive of the rate of future kidney function decline. The best biomarker to date is the total kidney volume (TKV). TKV (often adjusted for height and abbreviated as htTKV) has the merit of being a direct readout of cyst growth, which is the essence of the disease. TKV increases exponentially at a rate that is unique for each patient and averages 5% per year across the ADPKD population (18). The TEMPO 3:4 study provided the first evidence that the rate of

increase of TKV could be reduced by a drug and that this was associated with a salutatory effect on the rate of decline of kidney function (27). In the United States, TKV is not currently accepted by the Food and Drug Administration (FDA) as an outcome for the purposes of drug approval. However, the FDA did recently issue a Letter of Support for the use of TKV as a prognostic biomarker for enriching for patients with more rapidly progressive disease in clinical trials.

Long-term tolerability needed for drug acceptability

Finally, because the pathogenesis of ADPKD involves abnormal cell proliferation, resembling a benign neoplasm (28), many drugs currently in development for this disease are repurposed cancer therapies (29, 30). However, anticancer drugs are usually administered for short durations and, because of the acute life-threatening nature of the disease, even substantial toxicity can be considered acceptable. By contrast, therapy for ADPKD would likely need to be lifelong, and start when the patient is young and asymptomatic, so the bar in terms of drug tolerability and safety has to be set very high. This should influence the design of any clinical trial and the overall development strategy.

Ongoing or recently completed clinical trials of therapy

Blood pressure and the renin-angiotensin system

Hypertension is an early and major manifestation of ADPKD and is associated with accelerated progression to ESKD as well as increased cardiovascular morbidity and mortality (31, 32), as it is in other kidney diseases. Activation of the renin-angiotensin-aldosterone system (RAAS) has been shown to be a major contributor to the hypertension of ADPKD (33). There is also some evidence to suggest that the RAAS might directly promote cyst growth (34, 35), perhaps via its mitogenic effects. Inhibitors of RAAS such as ACEI are already standard of care for the treatment of hypertension in chronic kidney disease (CKD), but suppression of RAAS is incomplete. This is due to the existence of non-ACE pathways for angiotensin II generation, such as chymase, which has been shown to be upregulated in ADPKD (36). Thus, addition of an ARB to ACEI could potentially suppress the RAAS further.

To test these hypotheses, the Halt Progression of Polycystic Kidney Disease (HALT-PKD) Study Group designed two National Institute of Diabetes and Digestive and Kidney Disease (NIDDK)-sponsored concurrent, multi-center, randomized controlled trials (RCT), the HALT study A of 558 ADPKD patients with early disease (estimated GFR by the

MDRD equation, $eGFR > 60 \text{ mL/min/1.73 m}^2$), and the HALT study B of 486 patients with late disease ($eGFR 25\text{--}60 \text{ mL/min/1.73 m}^2$) (37). Study A tested whether low BP control (95 to 110/60 to 75 mm Hg) would delay progression of kidney disease compared to standard BP control (120 to 130/70 to 80 mm Hg), and both study A and B tested whether combination therapy with an ACEI and ARB (lisinopril + telmisartan) would delay progression of kidney disease compared to ACEI monotherapy (lisinopril + placebo).

In Study A (26), the primary outcome, the annual rate of growth of TKV, was 14.2% slower in the low BP group compared to the standard BP group (5.6% vs. 6.6%, $p=0.006$). The overall change in the $eGFR$ over the course of the study was no different between the groups. A pre-specified analysis of the short-term (0-4 months) and long-term (>4 months) effects showed that the low BP group as compared to the standard BP group experienced a short-term decline in $eGFR$ after starting treatment (-3.1 vs. $0.5 \text{ mL/min/1.73 m}^2$ per 4 months, $P<0.001$) which masked a beneficial effect on the slope of the $eGFR$ in the long-term phase that reached marginal statistical significance (-2.7 vs. $-3.1 \text{ mL/min/1.73 m}^2$ per year, respectively; $P = 0.05$). The low BP group had slightly more dizziness and light-headedness but no other adverse effects.

In both Study A and Study B (26, 38), there was no additional benefit of combination therapy with ACEI and ARB, as compared to ACEI monotherapy. This is consistent with several recent studies in other conditions that have shown that any benefits from dual blockade of the RAAS are outweighed by increased incidence of hypotension, AKI and hyperkalemia (39-41). Importantly though, in HALT-PKD combination therapy with an ACEI and ARB was not associated with excess adverse events, showing that combination therapy can be administered safely if needed in this population.

Vasopressin V2 receptor antagonists

Cyclic adenosine-3',5'-monophosphate (cAMP) plays a major role in driving cyst growth in PKD, by stimulating both fluid secretion and cell proliferation in cyst-lining epithelial cells (42). ADPKD cysts largely originate from the collecting duct, a nephron segment that expresses vasopressin V2 receptors (V2R) which signal through GS, adenylate cyclase and generation of cAMP. Thus, vasopressin, signalling through V2R, accelerates cyst growth. Moreover, one can surmise that cAMP is probably an early and central node in the signaling cascade that drives cyst growth because when Brattleboro rats that have genetic absence of vasopressin were bred with a strain of rats with polycystic kidneys (PCK), cystogenesis was almost completely inhibited (43). V2R antagonists have been tested in a number of rodent PKD models and found to be effective at retarding disease progression in all of them (44-46).

Based on these findings tolvaptan, a V2R antagonist already clinically approved for the treatment of hyponatremia, was tested in ADPKD. The Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and Its Outcomes (TEMPO) 3:4 trial was a multicenter, double-blind RCT (27). 1445 patients with ADPKD with a TKV of >750 mL and estimated creatinine clearance >60 mL/min were randomized to tolvaptan or placebo and followed for 3 years. The annual rate of change of TKV was reduced by half by tolvaptan, from 5.5% to 2.8%. Tolvaptan treatment was associated with a slower decline in kidney function (eGFR slope of -2.72 mL/min/1.73 m²/yr in the tolvaptan group versus -3.70 in the placebo group). Patients on tolvaptan had more adverse events related to increased aquaresis (thirst, polyuria, nocturia, and polydipsia), and 8% of patients in the treatment group discontinued the trial drug for this reason. More patients who received tolvaptan than those who received placebo (4.9% vs. 1.2%) had elevations of alanine aminotransferase to greater than 2.5 times the upper limit of the normal range. However, due to the design of the TEMPO 3:4 trial, the effects of tolvaptan on patients with more advanced ADPKD are not available.

In summary, tolvaptan convincingly slowed down ADPKD disease progression and reduced the rate of decline in eGFR by 1 mL/min/1.73 m²/yr which might be expected to delay the onset of ESRD significantly. Unfortunately, tolvaptan was poorly tolerated by some patients, and the increased incidence of abnormal liver function tests raised some concern that there might be a risk of serious hepatotoxicity associated with its use.

Largely on the basis of TEMPO 3:4, tolvaptan has now been approved for treatment of ADPKD in Japan, Canada and Europe. In the US, tolvaptan was not approved by the FDA. However, Otsuka is sponsoring another trial, REPRISE, which will be conducted under FDA guidance and will recruit patients with more advanced stages of CKD with the goal of obtaining more accurate estimates of the treatment effect on decline in kidney function and the incidence of hepatotoxicity.

Somatostatin analogs

Somatostatin is a peptide inhibitory hormone that signals via somatostatin receptors to inhibit the generation of intracellular cAMP. Somatostatin receptors are expressed in the kidney and liver, so somatostatin analogs might be expected to ameliorate cyst progression in both the kidney and the liver in ADPKD patients. In the ALADIN trial (A Long-Acting somatostatin on Disease progression in Nephropathy due to ADPKD), 79 patients with eGFR >40 mL/min/1.73 m² were randomized to receive, every 4 weeks, injections of octreotide-LAR, a synthetic analog of somatostatin encapsulated into microspheres for

long-acting release, or placebo injections, and were followed for 3 years (47). After 1 year, the increase in TKV was reduced by 2/3 with octreotide compared to placebo ($p=0.032$). Over the entire 3-year duration of the trial, the increase in TKV in the octreotide group was half that of the placebo group, but this was no longer statistically significant. This suggests either that the treatment effect is attenuated over time, or simply that the sample size was too small. 10% of patients treated with octreotide developed cholelithiasis or acute cholecystitis, raising concerns about its safety with long-term use.

Larger trials of somatostatin analogs are clearly needed. ALADIN 2 is an ongoing Phase 3, double-blind, placebo-controlled RCT being conducted in Italy with a planned recruitment of 98 patients. The design is similar to that of ALADIN except that patients with eGFR of 15-40 ml/min/1.73 m² are being enrolled, presumably to select for patients with rapidly declining GFR that might benefit more from the treatment. DIPAK 1 is a Phase 3 RCT in 300 patients conducted in the Netherlands that will compare open label lanreotide, administered subcutaneously every 4 weeks, with standard care (48).

Mammalian target of rapamycin (mTOR) inhibitors

The cytoplasmic tail of polycystin-1 has been shown to interact with tuberlin, and the mTOR pathway is inappropriately activated in cyst-lining epithelial cells in ADPKD (49). Inhibitors of mTOR are widely used clinically as immunosuppressants. In several rodent models of PKD, mTOR inhibitors have been shown to ameliorate the disease (13, 49-51). However, the clinical trials of mTOR inhibitors have been disappointing.

Walz et al. randomized 433 patients with ADPKD and an average eGFR of 53-56 ml/min/1.73 m² to receive the mTOR inhibitor, everolimus, or placebo in a double-blinded fashion, and then followed them for 2 years (14). The increase in TKV at 1 year was reduced by about 1/3 in the everolimus group compared to placebo. There was a similar reduction at 2 years but it was no longer significant. Despite the improvement in kidney volume, eGFR declined faster in the everolimus group than in placebo (5.5 ml/min/1.73 m² vs. 3.5 ml/min/1.73 m² respectively, $p<0.001$). The reason for this is unclear, but a number of possibilities have been proposed. First, the mTOR pathway mediates glomerular hypertrophy and hyperfiltration, thus maintaining GFR after nephron loss, so everolimus may have deprived the kidneys of this compensatory mechanism. Second, short-term changes in GFR do not necessarily correctly predict long-term changes and so 2 years of follow-up is probably too short to ascertain the true long-term trend. Indeed, if glomerular hyperfiltration is harmful in the long term, then it would be predicted that amelioration of hyperfiltration would reduce the decline in GFR given sufficient follow-up time. Third, as more patients on everolimus experienced edema and needed diuretics, this might have worsened kidney function. Finally, the selection of a patient population with relatively

advanced CKD and probably significant fibrosis and irreversible kidney injury may have obscured any potential benefit conferred by everolimus (52).

In a smaller, open label trial, Serra et al. randomized 100 patients to receive sirolimus or placebo (15) and found no difference in the rate of increase of TKV. The study was not powered to assess kidney function and so not surprisingly, eGFR did not differ significantly between the groups.

An additional consideration is that inability to administer sufficient dosage to achieve biological efficacy may have accounted for the lack of clinical efficacy of the mTOR inhibitors, as compared to the preclinical studies. As discussed earlier, the study by Canaud et al. suggested that sirolimus dosed as an immunosuppressant post-transplant is inadequate to inhibit mTOR activity in PKD cysts (16). However, higher doses are unlikely to be achievable. In the study by Walz et al. (14), one third of patients did not complete the study because of drug-related adverse events, including proteinuria which might adversely affect kidney function in the long-term. In the study by Serra et al. (15), the drug dose was lower than intended because of dose-limiting side-effects.

High water intake

Given the importance of vasopressin in the rate of cyst growth, it has been suggested that simply increasing water intake would be sufficient to suppress vasopressin secretion, and hence retard disease progression in ADPKD (53). Studies in PCK rats confirmed that increasing water intake by 3.5-fold retarded cyst growth and the decline of kidney function (54). Wang et al. developed a simple clinical method based on a measurement of urine osmolality to estimate the amount of additional water that should be ingested (55). However, many nephrologists are already recommending that their ADPKD patients empirically increase their fluid intake. The practice is widespread in the community, thus making it challenging to test the efficacy of high fluid intake in a randomized controlled trial. The only clinical study of high water intake that has so far been conducted was by Higashihara et al. They assigned 30 patients to high and free water-intake groups based on patient preference, and found that the slope of TKV and eGFR were worse in the high water intake group (56). However, the study was flawed because of the non-randomized assignment to the different groups, and was much too small to reach any meaningful conclusions.

Other therapies

Statins, or 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, have pleiotropic effects, including inhibiting farnesylation and activation of the monomeric

GTP-binding protein, Ras, and hence inhibiting cell proliferation (57). Statins have been shown to be effective in preclinical models of PKD (35,58). In a placebo-controlled RCT conducted in 110 children with ADPKD, pravastatin was shown to reduce the change in htTKV over 3 years (23% vs. 31%) (59). This is notable for being the first substantive therapeutic trial in ADPKD to be conducted in children.

The epidermal growth factor (EGF) receptor is overexpressed and mislocalized to the apical membrane in autosomal dominant and recessive forms of PKD (60, 61). Tyrosine kinase inhibitors that block EGF receptor catalytic activity and downstream mitogenic signaling appear to be effective at retarding disease progression in rodent PKD models (62, 63). Two tyrosine kinase inhibitors are currently undergoing early phase clinical trials in ADPKD (Table 1). A concern with this approach is that this antiproliferative strategy may not be sufficiently specific for PKD cyst epithelium and hence would have significant adverse effects in other proliferating tissues. While such side-effects might be acceptable in a drug being developed for short-term treatment of a life-threatening malignancy, tolerability is a major concern in a drug being developed for treating asymptomatic young adults with ADPKD over a large duration of their lifetime.

Sirtuin 1 (SIRT1) is a nicotinamide adenine dinucleotide-dependent protein deacetylase that was recently found by Zhou et al. to be a novel pathway responsible for cyst growth (64). SIRT1 was shown to promote cyst growth by deacetylation and increased phosphorylation of retinoblastoma protein which becomes inactive, enabling transcription of genes that mediate entry into the S-phase of the cell cycle (65) and also by inhibition of apoptosis via deacetylation of p53 (66), an important tumor suppressor protein. By promoting a base-exchange reaction at the expense of deacetylation, niacinamide serves as a noncompetitive inhibitor of SIRT1 (67). Niacinamide (also known as nicotinamide) is a water-soluble amide derivative of nicotinic acid (also known as niacin) and these two molecules represent the two major forms of vitamin B3. Administration of either high-dose niacinamide, a pan sirtuin inhibitor, or EX-527, a SIRT1-specific small-molecule inhibitor, delayed cyst growth and improved kidney function in two orthologous mouse models of ADPKD (64).

The dose of niacinamide that would likely be needed for clinical efficacy in ADPKD is 30 mg/kg/day (compared to the recommended daily dietary allowance of vitamin B3, which is 14 to 16 mg/day). Fortunately doses of niacinamide of this magnitude have already been tested both in adults and in children over 3 years and found to be safe (68). Niacinamide is classified as a dietary supplement, so if it is found to be effective in the treatment of ADPKD, it would not need approval by the FDA, and would be widely available to ADPKD patients at very low cost. Because of this, niacinamide is being tested in two early

phase clinical trials at the University of Kansas Medical Center (KUMC). These trials are designed to assess biological efficacy, as determined by the level of phosphorylation of Rb and acetylation of p53 in peripheral blood mononuclear cells, to estimate the clinical effect in terms of reduction in the increment of htTKV at 1 year, and to assess the tolerability and safety of niacinamide in this population.

Triptolide is an active diterpene found in the traditional Chinese medicine, Lei Gong Teng. It has been shown to induce intracellular calcium release in a mechanism dependent on polycystin-2. By restoring calcium signaling it seems to inhibit PKD cell proliferation and delay cyst growth (69). In orthologous mouse models of ADPKD, triptolide delayed disease progression (70, 71). The only published clinical study was an open label trial of 9 patients with ADPKD and proteinuria >1 g/day, which is rather unusual in this disease (72). Triptolide appeared to reduce proteinuria in this uncontrolled study. An RCT of triptolide was initiated at Nanjing University School of Medicine (ClinicalTrials.gov Identifier: NCT00801268) but is currently reported as being terminated because of a high rate of drop-out. Another trial being conducted at Shanghai Changzheng Hospital is currently recruiting.

Future directions

Clinical testing of therapies in ADPKD presents a number of daunting challenges, particularly in the US. These include the long lead time of disease progression before GFR falls predictably, failure of the FDA to accept TKV as a surrogate endpoint for drug approval, and the cost of large-scale clinical trials. The following are recommendations and predictions for future directions that may best address these issues.

We urge continued emphasis on repurposing already-approved drugs and testing dietary supplements. These have the advantage that there is already some knowledge about their safety, thus dramatically reducing the risk and cost of their development. In addition, even if the clinical trial data fall short of meeting FDA criteria for approval for the indication of PKD treatment, it may be sufficient to convince the nephrology community to use a drug off-label (especially if it is inexpensive), or to use a dietary supplement off the shelf.

Along those same lines, we believe that diet could be a major determinant in PKD disease progression. This includes not only water intake, but also sodium intake (73) and the balance of acid and base equivalents (74). At KUMC we are completing a pilot study to test the feasibility and acceptability of a diet low in salt and animal protein and enriched in fruits and vegetables, and its efficacy at reducing net acid excretion (NCT01810614).

Table 1. Summary of major interventional trials in ADPKD¹

Intervention	Mechanism of action ²	Trial name	Sponsor	ClinicalTrials.gov Identifier	Trial design ³	Status and outcome	References
Standard vs. low BP control	ACEI + ARB	HALT A	NIDDK	NCT00283686	2x2 factorial RCT in patients with eGFR ≥ 60 mL/min/1.73 m ²	Completed	(26)
Lisinopril vs. lisinopril + telmisartan							
Lisinopril vs. lisinopril + telmisartan	ACEI + ARB	HALT B	NIDDK	NCT01885559	RCT in patients with eGFR 25-60 mL/min/1.73 m ²	Completed	(38)
Tolvaptan	V2 receptor antagonist	TEMPO3/4	Otsuka	NCT00428948	Phase 3, placebo-controlled RCT in patients with eGFR ≥ 60 mL/min/1.73 m ²	Completed	(27)
Tolvaptan	V2 receptor antagonist	REPRISE	Otsuka	NCT02160145	Phase 3b, placebo-controlled RCT in patients with late stage 2 to early stage 4 CKD	Recruiting	
Octreotide	Somatostatin analog	ALADIN	Mario Negri Institute for Pharmacological Research	NCT00309283	Placebo-controlled RCT in patients with eGFR ≥ 40 mL/min/1.73 m ²	Completed	(47)
Octreotide	Somatostatin analog	ALADIN 2	Mario Negri Institute for Pharmacological Research	NCT 01377246	Phase 3, placebo-controlled RCT in patients with eGFR 15-40 mL/min/1.73 m ²	Active, not recruiting	

Lanreotide	Somatostatin analog	DIPAK 1	University Medical Centre Groningen	NCT01616927	Phase 3, placebo-controlled RCT	Active, not recruiting	(48)
Lanreotide	Somatostatin analog	LIPS	Assistance Publique - Hôpitaux de Paris/IPSEN Pharmaceuticals	NCT02127437	Phase 3, placebo-controlled RCT	Recruiting	
Everolimus	mTOR inhibitor		Novartis	NCT00414440	Phase 3, placebo-controlled RCT	Completed	(14)
Everolimus	mTOR inhibitor		Wyeth	NCT00346918	Phase 3, placebo-controlled RCT	Completed	(15)
Pulsed sirolimus	mTOR inhibitor	RAP	Medical University of Vienna	NCT02055079	Placebo-controlled RCT	Recruiting	
Pravastatin	HMG-CoA reductase inhibitor		University of Colorado, Denver/NIDDK	NCT00456365	Phase 3, placebo-controlled RCT in children	Completed	(59)
Bosutinib	Tyrosine kinase inhibitor		Pfizer	NCT01233869	Phase 2, placebo-controlled RCT	Completed	
KD019	Tyrosine kinase inhibitor		Kadmon	NCT01559363	Phase 1b/2a uncontrolled dose escalation study	Recruiting	
Niacinamide	Sirtuin inhibitor	NIAC-PKD1	University of Kansas Medical Center	NCT02140814	Phase 2, open label, single arm	Active, not recruiting	

Niacinamide	Sirtuin inhibitor	NIAC-PKD2	NIDDK	Pending	Phase 2, placebo-controlled RCT	Recruiting	
Triptolide	Stimulator of calcium release		Nanjing University School of Medicine	NCT00801268	Open label RCT	Terminated	
Triptolide	Stimulator of calcium release		Shanghai Changzheng Hospital	NCT02115659	Phase 3, placebo-controlled RCT	Recruiting	
Renal sympathetic denervation		RAFALE	Shanghai Changzheng Hospital	NCT01932450	Phase 2, open label RCT	Recruiting	

¹Studies were identified primarily from a search of the ClinicalTrials.gov database. Please note that this is not a comprehensive listing of all PKD trials. It includes primarily interventional studies where disease progression was an endpoint. Studies of patients that received kidney transplants, studies of effect on liver disease progression, observational studies, studies that were terminated and not published, and small early phase studies that were superseded by larger studies with more rigorous design are omitted.

²ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin AT₁ receptor blocker; mTOR, mammalian target of rapamycin; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A

³Specific details of study population are included only where needed to highlight the difference between two apparently similar trials.

Controlled trials that test the clinical efficacy of dietary intervention, while not particularly exciting, could have a major impact on the management of ADPKD.

Because ADPKD is a lifelong disease, we believe that early treatment has the greatest potential to change its long-term course and so treating children is likely to have the greatest benefit (20). Clinical trials are rarely conducted in children because of concerns about safety and ethics but they are certainly possible, and therapies such as niacinamide that have already been shown to be safe in children (68) could lead the way.

Because falling GFR is a late event in ADPKD and an indicator of irreversible, fibrotic changes within the kidney, earlier biomarkers of disease progression that reverse in response to effective therapy are badly needed. While a number of blood and urine biomarkers have been identified (75-81), none has yet been shown to have the sensitivity and reliability needed. One promising new area of biomarker discovery is that of urinary exosomes, which are a rich source of renal tubule epithelium-derived proteins and, particularly, of proteins that are complexed to the polycystins (82).

Another approach might be to test therapies in a cystic kidney disease with a more rapid and aggressive course. Because the pathogenesis of autosomal recessive PKD (ARPKD) closely resembles that of ADPKD, testing therapies in children with ARPKD, who generally have a rapid decline in kidney function in childhood, might enable one to see a change in the GFR slope early on. Even detecting a change in the incidence of a hard outcome, such as ESRD, might well be feasible. Moreover, ARPKD (unlike ADPKD) is unequivocally a rare disease and as there is also no currently approved treatment, any candidate drug would be considered an orphan drug and qualify for regulatory and financial incentives that would greatly facilitate its commercial development (83).

Rigorously designed and adequately powered late-stage trials to definitively prove the clinical efficacy of a therapy requires large numbers of patients and, particularly for off-patent drugs and for therapies that cannot be commercialized, such as dietary interventions and dietary supplements, the cost may be prohibitively expensive. Pragmatic trials that test therapies in real-world clinical settings using the existing infrastructure and protocols of standard care may allow such trials to be conducted at much lower cost and may even be better at predicting their real clinical effectiveness (84).

Conflict of interest

M.E. serves as a study investigator on clinical studies conducted at KUMC sponsored by Otsuka, Kadmon and Genzyme. F.T.W. has served as a study investigator on clinical studies conducted at KUMC sponsored by Otsuka.

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Chapter 7

Polycystins and Molecular Basis of Autosomal Dominant Polycystic Kidney Disease

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is the most common renal monogenic disorder. It is characterized by progressive, bilateral renal cystic expansion followed by gradual loss of renal function after decades of life, while its systemic nature is reflected by extra-renal manifestations typically involving liver and the cardiovascular system. Cyst formation is triggered by mutations in the *PKD1* or *PKD2* genes. In most cysts, if

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not all, cystogenesis follows a two-hit model in developmental kidneys, while in the mature organ broad and fast cyst formation requires a third hit such as kidney injury. The first hit is represented by the germline mutation whereas a somatic total or partial inactivation of the previously normal allele constitutes the second event, a process that is consistent with the focal nature of ADPKD cystogenesis. *PKD1* encodes polycystin-1 (PC1), a likely transmembrane mechano-sensor receptor comprised of a singular combination of structural domains present in other proteins. *PKD2*, in turn, encodes polycystin-2 (PC2), a non-selective cationic channel permeable to calcium composed by six transmembrane helices and intracytosolic C- and N-termini. In the primary cilium, PC1 regulates cell calcium influx by physically interacting with PC2 through their intracytosolic domains. Disruption of calcium cellular homeostasis increases cAMP cytosolic levels and affects cell cycle, leading to increased cell proliferation and transepithelial fluid secretion. In addition, disruption of PC1 C-terminus interactions with components of Wnt, mTOR, STAT3 and JAK2/STAT1 pathways is translated into a number of intracellular pathway abnormalities. In the same line, interactions between the PC2 C- and N-termini with ERK/B-Raf, GSK3 β and other partners lead to disturbed cell proliferation, apoptosis and cell polarity. Mutations in *PKD1* can result, moreover, in cell adhesion and extracellular matrix alterations, due to the role of PC1 extracellular domains in cell-cell and cell-matrix contact. Defects in the mentioned pathways can also impact oriented cell division, contributing to cyst growth.

Key words: ADPKD; Cystogenesis; *PKD1*; *PKD2*; Polycystin-1; Polycystin-2

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic renal disease, presenting a worldwide prevalence of 1:400-1000 (1). ADPKD is characterized by an intense and bilateral development of renal cysts that leads to progressive organ enlargement and, eventually, loss of renal function. This entity constitutes a systemic disorder, comprising cystic and noncystic involvement of multiple organs, including liver, pancreas, blood vessels, heart and brain (2). ADPKD is a genetically heterogeneous disease originated by a germline mutation in one of two genes, *PKD1* or *PKD2* (Polycystic Kidney Disease 1 and 2) (3,4). While *PKD1* is responsible for 85% of the cases in clinically-affected families (ADPKD1) and is associated with a more severe clinical course, mutations in *PKD2* are present in the remaining 15% of the patients (ADPKD2), who generally present a milder renal functional decline and a lower renal complication rate. Indeed, the progression to end-stage kidney disease (ESKD) occurs at the mean age of 54 years for ADPKD1 affected individuals and 74 years for ADPKD2 patients (5). This difference, however, is not sufficient to distinguish the two forms clinically, since the disease is associated with a large intra- and

inter-family phenotypic variability (6). Given its slower progression to ESKD, the lack of diagnosis in asymptomatic individuals suggests a likely underestimation of the ADPKD2 true prevalence. In fact, a population study revealed an ADPKD2 prevalence of 26% (7). Additionally, *PKD1* and *PKD2* mutations may coexist in the same patient, leading to severe and early ESKD, evincing interaction between these genes (8). A third ADPKD locus was suggested based on previous linkage analyses, however further studies performed in some of such families disregarded this possibility (9).

The *PKD1* and *PKD2* genes and products

PKD1 is a 46-exon gene localized at 16p13.3, spanning a genomic region of approximately 52-kb and giving rise to a 14-kb mRNA (3). In addition to its large size, six pseudogenes highly homologous to its 5' portion are also positioned on chromosome 16, sharing up to 99% of sequence identity. This peculiarity makes *PKD1* sequencing a challenging task and turns the employment of direct mutation analysis in the clinical setting more complex. *PKD1* encodes polycystin-1 (PC1), a transmembrane likely mechano-sensor receptor that encompasses a unique combination of structural motifs present in other proteins of known functions (Figure 1). Such features give a versatile structure to PC1, with the ability to perform a large number of functions and to interact with several molecular binding partners.

The N-terminus of the PC1 polypeptide chain starts with a signal peptide and leucine-rich repeats (LRR), a motif responsible for its interaction with the extracellular matrix and cell adhesion (Figure 1). The following domains are the cell wall integrity and stress response component (WCS) domain, thought to interact with carbohydrates, and a type-C lectin motif, involved in biological processes such as cellular signaling and exocytosis. An LDL-A domain follows along the primary sequence, suggesting a possible interaction between PC1 and LDL-related molecules. At the central portion of the chain, PC1 has 16 PKD repeats that share sequence similarity with immunoglobulin-like and fibronectin type-3 domains. Such repeats are supposedly stabilized by force-induced formation of a stable intermediate state, which is consistent with a role for PC1 in mechanical coupling between cells (10). The next motif is the polycystin-1 lipoxigenase α -toxin (PLAT) domain, the most conserved motif and the family signature of the PC1-like proteins. This domain has been identified in more than 1000 proteins with functions related to lipid binding (often calcium-dependent) and, sometimes, to protein binding. In MDCK (Madin-Darby Canine Kidney) cells, however, PLAT targets PC1 to the plasma membrane in a selective binding process to phosphatidylserine and L- α -phosphatidylinositol-4-phosphate (PI4P). This event is regulated by protein kinase A (PKA)-mediated phosphorylation of the PLAT domain,

which reduces PI4P binding and recruits β -arrestins and clathrin adaptor AP2 to trigger PC1 internalization (10). Just before the first transmembrane helix comes the GAIN (G protein-coupled receptor-autoproteolysis inducing) regulatory domain. The functional role of the GAIN-mediated PC1 cleavage is yet not well understood; nevertheless, a *Pkd1* GPS cleavage mouse mutant escapes lethality but develops rapidly progressive postnatal PKD (11). At this point, the PC1 polypeptide chain comprises 11 transmembrane domains, passes to the cytoplasm, and ends with the G-protein binding and the coil-coiled domains.

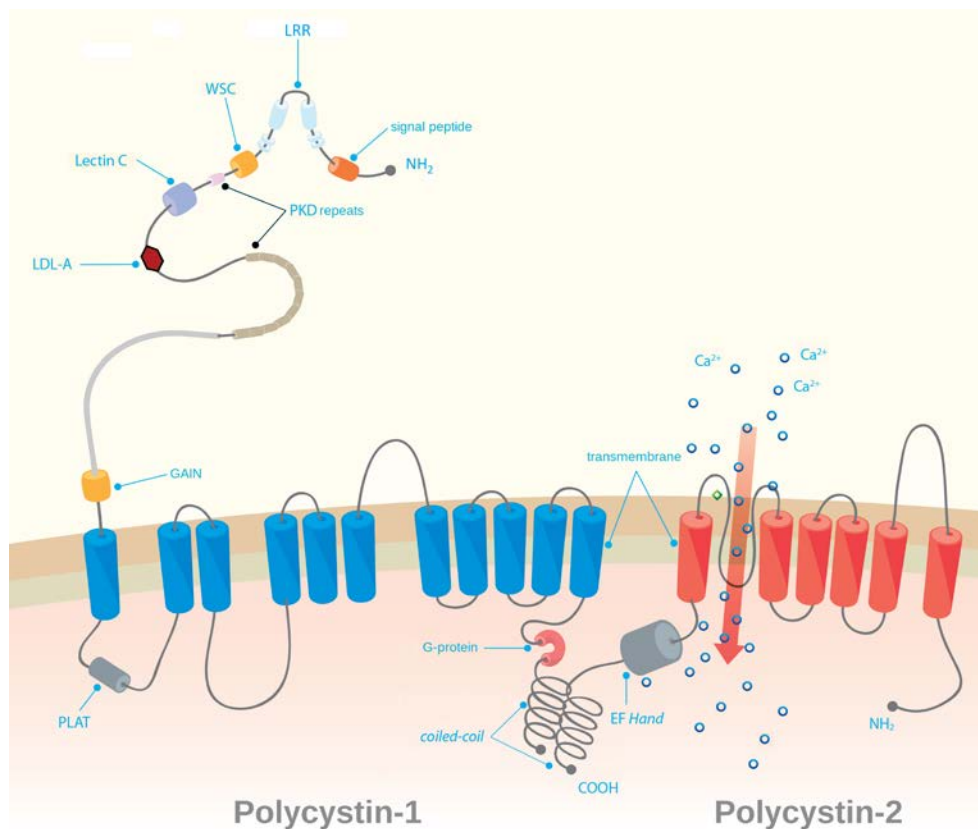


Figure 1. Domain structure of polycystins. Polycystin-1, from N- to C- terminus: signal peptide; leucine-rich repeats (LRR); WSC domain; lectin C type-3 domain; low-density lipoprotein-A domain (LDL-A); polycystic kidney disease (PKD) repeats; protein-coupled receptor auto-proteolysis inducing (GAIN) domain; Polycystin-1, Lipoxigenase, α -Toxin (PLAT) domain; transmembrane domains; G-protein domain; and coil-coiled domain. Polycystin-2, from N to C terminus: transmembrane domains; helix-loop-helix motif, calcium-binding EF-hand domain; and coil-coiled domain.

PKD2 localizes on 4q21, is smaller and encompasses 15 exons (4). However, as for *PKD1*, it presents high allelic heterogeneity and pathogenic mutations distributed along the gene (12). Its product, polycystin-2 (PC2), is a member of the transient receptor potential (TRP) superfamily, functioning as a non-selective cation channel of six transmembrane helices with high permeability to ionic calcium (Figure 1). Similarly to PC1, PC2 presents an intracytoplasmic C-terminal portion including a calcium-binding EF-hand and a coil-coiled subdomain which interacts with PC1 C-terminus and a number of additional binding partners (13) (Figure 1). In contrast, the PC2 N-terminus is small (223 amino-acids) and is turned to the cytosol.

The PC1 and PC2 molecular features, associated with their expression profiles, support the systemic nature of ADPKD. It must be noted that truncation mutations predominate in ADPKD, accounting for around 65% of the ADPKD1 and 83% of the ADPKD2 cases (12,14). Point mutations, in turn, are frequently found to be of uncertain clinical significance.

An interesting genomic aspect of *PKD1* is its tail-to-tail chromosomal positioning to the *TSC2* gene (Tuberous Sclerosis Complex 2). Contiguous deletions involving both loci lead typically to an ADPKD-TSC associated phenotype expressing severely cystic kidneys (15). Such patients usually progress to early ESKD by the third decade of life.

PKD1 and *PKD2* also interact with genes mutated in various renal cystic diseases. All typical forms of autosomal recessive polycystic kidney disease (ARPKD) are caused by mutations in the *PKHD1* (Polycystic Kidney and Hepatic Disease 1) gene. Mice homozygous for a *Pkhd1* (the mouse orthologue to *PKHD1*) hypomorphic allele (*Pkhd1*^{del3-4}) and heterozygous for a *Pkd1*-null mutation showed a more severe renal cystic phenotype than *Pkhd1*^{del3-4/del3-4} animals (16). This genetic interaction was later extended to a genetic network, by breeding strategic genetically-modified mice with *Pkd1*, *Pkd2*, *Pkhd1*, *Sec63* and *Prkcsh* mutant alleles. The latter two genes are the mouse orthologues to human genes mutated in autosomal dominant polycystic liver disease (ADPLD) (17). The combination of *Sec63* or *Prkcsh* loss of activity with *Pkd1* or *Pkd2* null heterozygosity led to remarkable exacerbation of renal cystic burden (18). Loss of *Sec63* function associated with *Pkhd1* hypomorphic homozygosity also led to worsening of the renal cystic phenotype. This same study, in turn, revealed that PC1 overexpression was able to rescue the phenotype of cystic mice generated by inactivation of other than *Pkd1* genes. In this setting, the comprehensive analysis of all breedings allowed the proposal of a genetic network, based on the hypothesis that PC1 is the central determinant of cystic phenotype severity. Interestingly, *Sec63* and *Prkcsh* are genes involved in translocation and quality control of proteins in the

endoplasmic reticulum (ER), supporting PC1 processing as a limiting step in cyst development.

PC1-PC2 interactions and assembly

The interactions of polycystins between themselves or with other binding partners are of major interest and have been subject of a large number of studies. Such investigations are essential to determine and elucidate relevant biological pathways involved in the disease, as well as to identify potential treatment targets.

The most important and studied PC1 binding partner is PC2. This interaction has been described at several levels, from single amino acid contacts, passing through domain-domain interaction (19), to homo- and hetero-oligomeric associations (20,21). This binding process has been largely scrutinized by different experimental and computational approaches, using both *in vitro* and *in vivo* systems. Most of them point to the PC1 and PC2 intra-cytosolic domains as the critical interacting regions responsible for driving homo- and hetero-assembly. The EF-hand and the coil-coiled subdomains, in particular, represent the most important regions and constitute the key elements to understand the protein oligomerization state, as well as how they interact to each other. In the described scenario, the PC1 and PC2 homo- and hetero-interactions became a central and controversial issue in the PKD research field. PC2 seems to be required for the proper processing and trafficking of PC1 (22). Disruption of PC1-PC2 interaction, in fact, precludes PC1 to reach its mature glycosylated isoform needed to target the primary apical cilium (PAC, Figure 2). PC2 trafficking to the PAC, on the other hand, is not dependent on PC1, since the truncation of PC1 C-terminus does not impair this process (23). Transfection experiments with human embryonic kidney cells (HEK293T) showed that PC2 selectively associates with the transient receptor potential channels type 1 (TRPC1), but not type 3, to modulate calcium transient currents (19). In this study, a segment of 73 amino-acids within the PC2 C-terminus was shown to be one of the interacting regions. The fact that PC1-PC2 interactions are disrupted by native disease mutations supports biological relevance for a complex formed by these counterparts. The PC1 R4227X mutation, in fact, reduced the capacity of PC1 to interact with PC2 and abrogated calcium currents (24). Similarly, the deletion of the last 227 C-terminal amino-acids caused by the PC2 R742X mutation also led to calcium current disruption. Interestingly, this study suggested the need of PC1 to mobilize PC2 to the plasma membrane and regulate the intracellular calcium homeostasis (24).

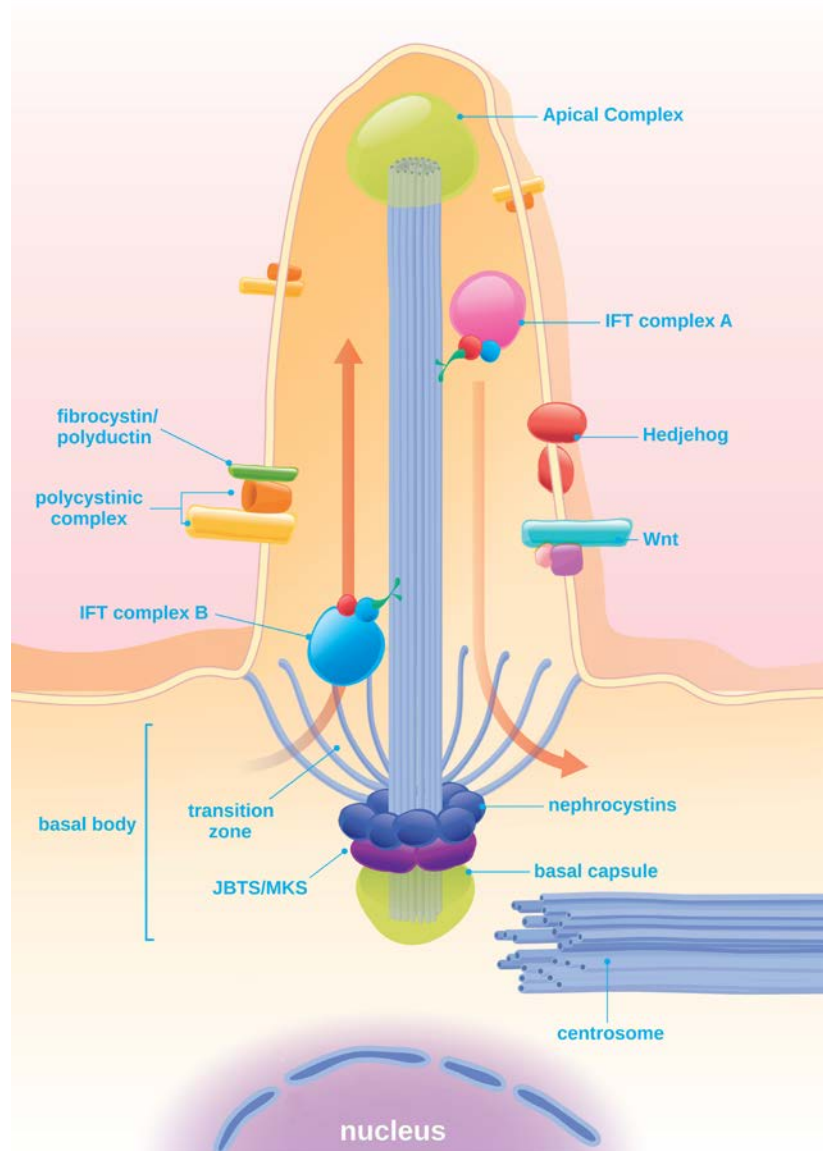


Figure 2. Basic structure of the primary apical cilium. The basal body separates PAC from the cytoplasm. This structure controls the ciliary trafficking in the transition zone and anchors the axoneme at the centrosome. JBTS (Joubert syndrome), MKS (Meckel syndrome) and nephrocystins are components of the basal body. IFT-B and IFT-A, respectively, transport protein cargo to and from the axoneme tip. PC1/PC2/FPC form a complex involved in calcium transients. FPC: fibrocystin/polyductin. Wnt (wingless integration 1) and Sonic Hedgehog are additional pathways present in the PAC.

Biophysical and biochemical studies addressed the multimerization properties of the polycystins. Atomic force microscopy (AFM) is a high resolution technique capable of producing images of big complexes and oligomers at a molecular level, such as receptors and channels. AFM imaging and single-channel patch clamp experiments supported a homo-tetrameric structure for PC2, a finding consistent with the PC2-expected functional roles (25). Other atomic force microscopy studies with PC1 and PC2, isolated from singly-transfected tsA 201 cells, indicated not only assemblies of PC1 and PC2 homotetramers, but also PC1-PC2 heterodimers with a 2:2 subunit stoichiometry following an alternative arrangement (26,27). The tetrameric arrangement is also found in other members of the TRP cation channel superfamily, such as TRPV6 (28) and TRPC1 (29). In support to this assembly pattern, a recent study reported a heteromeric TRP channel formed by TRPV4, TRPC1 and PC2 subunits (30).

Small Angle X ray Scattering (SAXS) analyses were also applied to investigate this theme. This technique takes advantage of X ray scattered by molecular surfaces. It has a wide applicability despite its low-resolution, ranging from nanoscience to soft condensed matter studies. The major advantage of SAXS over macromolecular crystallography is not to require crystals to derive molecular information, since the data are collected directly from protein solution (31). This technique, however, is sensitive to protein aggregation. In this context, SAXS has been successfully used to measure size, shape and oligomerization state of macromolecular complexes in solution. Based on the aforementioned principles, a study comprising SAXS and biochemical assays analyzed PC2, supporting a homo-tetrameric arrangement for the PC2 C-terminal intra-cytosolic domain. Such experiments also showed that the tetrameric organization does not depend on any other portion of the protein (32,33). A crystallographic study, on the other hand, supported a trimeric oligomerization for the coiled-coil subdomain of PC2 C-terminus (34), while a combined technical strategy supported this assembly for the whole PC2 C-terminus (35). Macromolecular crystallography is a very high resolution technique used to elucidate molecular structures. Its application requires molecular model building based on experimental X ray diffraction data from monocrystals (36). An additional resonance nuclear magnetic study conducted with the C-termini of PC2 and PC2-like channels also reached a trimeric arrangement (37). A similar structural organization was suggested by Size Exclusion Chromatography followed by Multi Angle Light Scattering (38), a very low resolution technique based on light scattering over the molecular surface. In this technique, the angle between the incident and scattered beams can be varied to increase the accuracy of the molecular hydrodynamic radius estimation. This information is usually introduced in the Stokes-Einstein equation and fitted against the experimental scattering intensity curve decay. This process involves assumption of a mono-modal gyration radius distribution and does not tolerate large deviations from globularity (39). Based on the available technical and

experimental information, both the trimeric and tetrameric PC2 C-terminus conformations are possible to occur *in vitro* and perhaps may coexist under specific conditions. Nevertheless, the trimeric arrangement seems less likely to be relevant *in vivo*, given that PC2 channel activity has not yet been shown for this conformation.

Polycystins in the cellular environment and the primary apical cilium

Discoveries in many renal cystic diseases and PKD animal models converged to the involvement of the primary apical cilium in cyst development (Figure 2). The observation of a common path for a number of such diseases gave rise to the concept of “ciliopathies” (40,41), a subset of disorders that include ADPKD, ARPKD, nephronophthisis, Bardet-Biedl syndrome and Meckel syndrome, among others.

PAC consists in a sensing organelle present in almost all nucleated cells in vertebrates (42) (Figure 2). It is a long projection of the plasma membrane, containing a microtubule-organized axonema running in its length and anchored in the centrosome. The PAC is separated from the cellular cytoplasm and membrane by a structure defined as basal body, which allows the cilium to maintain a unique composition of membrane proteins and to modulate signaling pathways differently from the rest of the cell.

Functional features

The PC1-PC2 complex, located at the PAC surface, appears to function as a fluid shear sensor to regulate the calcium signal. The PC1 extracellular portion is thought to be responsible for the mechano-sensor property, promoting the transduction of extracellular signal to the cytoplasm by activating PC2 through their intracytoplasmic domains. PC2, in turn, triggers the intracellular calcium release from the endoplasmic reticulum stores by modulating the inositol trisphosphate and ryanodine receptors (43,44). This regulation has been documented in mouse kidney cells. Cells bearing a *Pkd1* null mutation were unable to translate the shear stress signal into intracellular calcium transients. It must be noted, however, that a PC1L1-PC2L1 hetero-oligomeric complex has been recently shown to behave as the main calcium channel within the primary cilia in several cell types (45).

Another piece of work in cystic cells revealed that the loss of both ciliary polycystins led to defective calcium transients in response to angiotensin II and vasopressin (44). Ciliary disturbance has also been associated with cell division abnormality and incorrect planar cell polarity, suggesting that disoriented cell division may result in irregular tubule diameter and contribute to cyst development (46).

Binding partners and cell expression

Some studies showed that PC2, kinesin-2 and fibrocystin FPC (the *PKHD1* gene product), form a complex in the primary cilium, and in the perinuclear cytoplasm of renal epithelial cells (47,48). FPC stimulates PC2 channel activity in the presence of the kinesin-2 motor subunit, KIF3B, though the function of this complex is not completely understood (49). Notably, *Kif3A* knockout renal tubular epithelial cells do not have PAC, presenting cleavage of PC1 C-terminus and subsequent translocation to the nucleus to initiate AP-1 signaling (50). This pathway regulates gene expression in response to a variety of stimuli, including cytokines, growth factors and stress signals.

In a lipid bilayer electrophysiology system, α -actinin was shown to modulate the PC2 channel activity (51). In addition, PC2 and Hax-1 were shown to co-localize in the cell body of different cells. Hax-1 is a protein associated with the actin cytoskeleton, which binds the F-actin-binding protein cortactin to mediate association with other actin-binding proteins (52). PC2 was also found to directly associate with actin microfilaments of tropomyosin-1, a protein present in muscle cells (53), as well as to interact with an angiogenesis inhibitor, troponin-1 (54). Interestingly, PC2 was demonstrated to inhibit stretch-activated ion channels (SAC) and interact with filamin A, an actin crosslinking protein critical for SAC regulation (52). Yeast two-hybrid screening and immunoprecipitation assays revealed, in addition, that both PC1 and Pacsin2 C-termini domains and the neural Wiskott-Aldrich syndrome protein (N-Wasp) interact to form a protein complex (55). This complex, in turn, modulates the Arp2/3 protein complex function, involved in cell migration and actin filament nucleation, therefore contributing to the establishment and maintenance of the tubular architecture.

Mechanisms of cyst formation and growth in ADPKD

Renal cystic formation is a complex and not fully understood process in ADPKD, however it is known to involve cell clonal proliferation, increased apoptosis, abnormal epithelial cell phenotype, extracellular matrix alterations and inflammation (56). Though most cysts appear to derive from the collecting ducts, they may arise from any nephron segment. The development of such lesions follows a focal pattern, affecting less than 1% of the nephrons (57). The combination of the cited conjunctions appears to lead to focal tubular bulging, followed by the detachment of such a structure from the original nephron when it reaches a certain size. The continuous cyst expansion leads to progressive enlargement of the kidneys, alterations of their architecture and increase in kidney fibrosis; after reaching large kidney volumes, renal function loss occurs at a fast rate (56).

The two-hit, three-hit and threshold models

Although ADPKD presents dominant inheritance, its mechanism of cystogenesis is recessive at the cellular/molecular level, for most if not all cysts. Following a Knudsonian pattern, cyst formation was shown to obey a two-hit model in ADPKD human kidneys (58,59). The germline mutation constitutes the first event while the second hit is represented by a somatic mutation in the previously normal allele. According to this model, cystogenesis requires the inactivation or severe reduction of functional activities of both alleles of *PKD1* or *PKD2*. These observations are in accordance with the proposed clonal nature and focal profile of the cysts. They are also in agreement with the broad clinical variability within some ADPKD families and within the kidney itself, as well as with the increase in cyst number with age.

More recently, studies employing orthologous mouse models showed that a third hit is required for rapid and severe cyst development in mature kidneys. The inactivation of both *Pkd1* mouse alleles before 13 days of life led to massive and rapid cystogenesis, while after this age the same maneuver was followed by minor early cyst development and only late significant cystic disease (60,61). Interestingly, in another study both copies of *Pkd1* were inactivated at five weeks of age and, after three weeks, the mice were submitted to a unilateral renal ischemia-reperfusion insult (62). Remarkably, the injured kidney developed rapid and broad cystogenesis while the contralateral one did not, supporting the need for a third event for rapid cystogenesis in mature kidneys. The current thought is that this process depends on the reactivation of renal developmental programs and/or increase in cell proliferation rates triggered by renal injury. Altogether, the available studies suggest that cystogenesis arises when the *PKD1* or *PKD2* functional activity falls below a critical level in a given renal cell. This threshold model by Gallagher et al. (63) proposes, however, that this critical level can vary according to a series of factors, including the kidney developmental stage, environmental effects, increased functional demands induced by renal lesion, and genetic variants at modifier loci. Other cystogenic mechanisms have been described, however their relevance in human ADPKD has not been fully determined yet.

Role of defective intracellular calcium homeostasis and cell response to cyclic AMP

Intracellular calcium participates in a number of cell signaling pathways, including the regulation of cyclic adenylyl-monophosphate (cAMP) levels (64,65) (Figure 3). Low intracytosolic calcium leads to activation of adenylate cyclase 6 and inhibition of phosphodiesterases 1 and 3, favoring intracellular accumulation of this second messenger (66,67). As predicted by the molecular features of the polycystins, disruption of this pathway was shown to lead to a defective calcium homeostasis in renal tubular cells. Lack

of activity of either PC1 or PC2, in fact, impairs the calcium transient response determined by the PAC bending (68). Heterologous expression of PC1, in turn, inhibits calcium leakage through the ER membrane in MDCK cells. The high level of cellular cAMP detected in a number of ADPKD cells and animal models agrees with such findings (66,69,70). Interestingly, this calcium defective regulation also disrupts cell response to cAMP. While in normal cells cAMP induces cell cycle arrest, in ADPKD cells it leads to an abnormal proliferative response (71,72). cAMP has also been shown to participate in other components of ADPKD pathogenesis, such as inflammation, alterations in the cell polarity and, probably, extracellular matrix defects (68).

Increased cell proliferation and apoptosis

Clonal cell expansion is a major player in cyst development. Various pro-proliferative pathways are in fact upregulated in ADPKD. The significantly higher cyst epithelial cell number compared to renal tubules, observed in scanning electron micrographs, underscores this point (72). In support to this finding, several studies identified activation of the MAPK (mytogen-activated protein kinase)/ERK (extracellular-regulated protein kinase) pathway in cellular and animal PKD models (73–75) (Figure 3). This cascade transduces extracellular signal by activating small G proteins (SGP) which, in turn, lead to RAF1, LAMTOR3 and MAP3K phosphorylation. This sequential activation modulates the activity of transcriptional factors, favoring the advance of cell cycle. The inappropriate activation of this pathway in ADPKD is attributed to the accumulation of cAMP and consequent activation of PKA, apparently resulting from the defective calcium cellular handling (76). This abnormal signaling scenario activates phosphatidylinositol 3-kinase, resulting in the SGP activation (71) (Figure 3).

PKA activation also leads to tuberin (the *TSC2* gene product) phosphorylation, promoting upregulation of the mTOR (mammalian target of rapamycin) pro- proliferative pathway (77) (Figure 3). This process also appears to be responsible for the abnormal shift of glycolysis activation and intracellular ATP accumulation, favoring liver kinase B1 and AMP kinase inhibition and consequently further activation of mTOR (78). Notably, the PC1 intracytoplasmic tail directly interacts with tuberin, leading to mTOR inhibition. This may be another source of mTOR hyperactivation in ADPKD (79). PKA also contributes to cell proliferation by stabilizing β -catenin and inhibiting its degradation, which leads to canonic activation of the Wnt pathway. In addition, PKA activates CREB (cAMP responsive element-binding protein), promoting activation of the pro-proliferative STAT3 (signaling transducer and activator of transcription 3) (Figure 3) and PAX2 (paired box 2) pathways (80–82). Disruption of the JAK (Janus kinase)/STAT pathway has also been suggested to contribute to increased cell proliferation, since activation of PC1 activates JAK2 in a

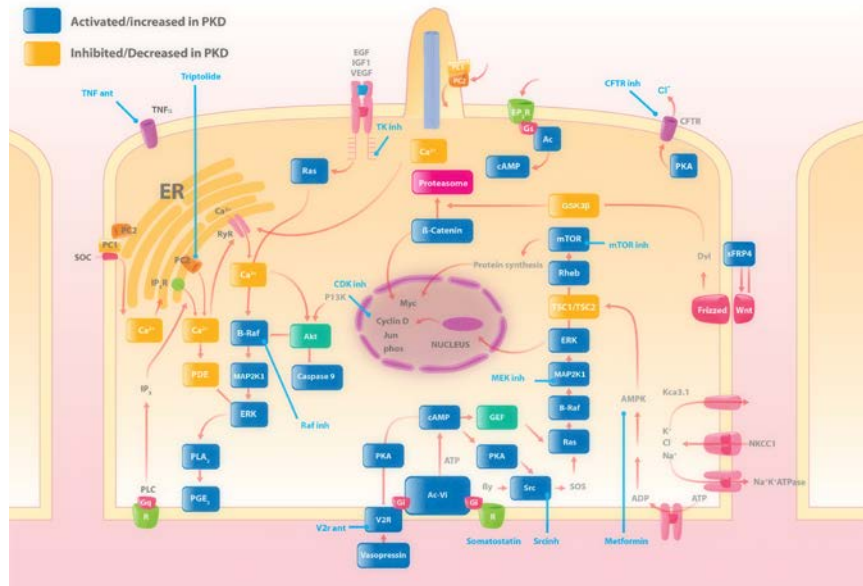


Figure 3. Cellular pathways involved in ADPKD pathogenesis. PC1 or PC2 loss of function impairs the calcium transient response mediated by PAC and ER, resulting in intracellular cAMP accumulation. An abnormal cell response to cAMP determined by the defective calcium homeostasis, in turn, leads to a pro-proliferative cellular status with activation of B-RafMAP2K1/ERK. Other alterations include loss of planar cell polarity with Wnt dysregulation and altered cellular energy metabolism, encompassing AMPK and mTOR disturbed modulation. The NKCC1 and CFTR localization pattern is essential to the observed secretory cell profile. V2R activation is a major source of cAMP production in the cell, consisting in a therapeutic target in ADPKD. TNF- α is a potential inflammatory mediator of the PKD phenotype. Adenylate cyclase (AC); adenosine monophosphate kinase (AMPK); v-raf murine sarcoma viral oncogene homolog B (B-Raf); cyclic adenosine monophosphate (cAMP); Cyclin-dependent kinase (CDK); cystic fibrosis transmembrane conductance regulator (CFTR); disheveled (Dlv); epithelial growth factor (EGF); E-prostanoid receptor 2 (EP2R); insulin-like growth factor (IGF1); endoplasmic reticulum (ER); extracellular signal-regulated kinase (ERK); guanine nucleotide exchange factor (GEF); G-protein inhibitory α subunit (Gi); G-protein q α subunit (Gq); G-protein stimulatory α subunit (Gs); inositol-triphosphate (IP3); glycogen synthase kinase 3 β (GSK3 β); mammalian target of rapamycin (mTOR); Na-K-Cl cotransporter (NKCC1); Polycystin-1 (PC1); Polycystin-2 (PC2); phosphodiesterase (PDE); prostaglandin (PGE2); phospholipase A2 (PLA2); Phospholipase C (PLC); G-protein coupled receptor (R); rat sarcoma (Ras); Ras homolog enriched in brain (RHEB); ryanodine receptor (RyR); store-operated channel (SOC); secreted frizzled-related protein 4 (sFRP4); son of sevenless (SOS); sarcoma (Src); tumor necrosis factor α (TNF α); tuberous sclerosis complex 1 (TSC1); tuberous sclerosis complex 2 (TSC2); vasopressin 2 receptor (V2R); vessel endothelial growth factor (VEGF); wingless integration 1 (Wnt).

PC2-dependent manner. This process results in phosphorylation and formation of STAT1 homodimers, their translocation to the nucleus, p21 upregulation and inhibition of Cdk2 (cyclin-depend kinase-2) activity (83). In support to this mechanism, experiments conducted in the PKD mouse models *jck* and *cpk* showed that the administration of roscovitine, a Cdk inhibitor, resulted in inhibition of cystic disease (84). In parallel to high proliferation rates, ADPKD is associated to increased apoptosis. As predicted, PC1 has an anti-apoptotic effect. Pull-down experiments and NMR (nuclear magnetic resonance) structural studies showed association between the PC1 polyproline motif and the nephrocystin-1 (NPHP1) SH3 domain (85). In this system, PC1 requires NPHP1 to regulate resistance to apoptosis, but not to regulate cell cycle progression. Additionally, PC1 is capable to direct association with G proteins and, therefore, to participate in apoptosis regulation. Interestingly, a recent study reported that the induction of tumor necrosis factor- α (TNF- α)-dependent cyst epithelial cell apoptosis using a second mitochondria-derived activator of caspase (Smac)-mimetic in a *Pkd1*-targeted model led to attenuation of cyst development, bringing a different potential perspective for the role of apoptosis in ADPKD (86).

Alterations in cell polarity and fluid secretion

Cell polarity is essential for proper tubular cell function, by allowing the insertion of different sets of membrane transporters in the basal and apical membranes. The loss of this arrangement disrupts the appropriate flux of ions and water, turning the tubular epithelium pattern from absorptive to secretory. The localization of a Na-K-2Cl cotransporter in the basolateral membrane, along with the expression of cystic fibrosis transmembrane conductance regulator (CFTR) in the apical membrane (87), represents key abnormalities responsible for chloride and fluid secretion in ADPKD cysts.

Planar cell polarity is also disturbed in ADPKD. This alteration is attributed to *Wnt* canonical activation accompanied by reduction in non-canonic Wnt activity. Inappropriate spatially-oriented cell division (OCD), in turn, hinders the maintenance of tubule structure, favoring tubular dilation (88). Interestingly, in mouse models orthologous to ADPKD loss of OCD was not present before cystic formation, while *Pkhd1* deficient mice did not develop renal cysts despite loss of OCD. These data indicate that alterations of planar cell polarity are not the primary cause of cyst formation (89).

Role of inflammation in cyst growth and renal fibrosis

A number of studies support a role for inflammation in ADPKD cyst development. The observations that a PKD mouse non-orthologous to human ADPKD develops a smaller

number of renal cysts when kept in a germ-free environment, and that endotoxins are capable of rescuing the cystic phenotype in a chemically-induced PKD rat model, underscore this point (90,91). In addition, other studies in human ADPKD and rodent cystic models reported high levels of cytokines and chemokines in the cystic fluid as well as interstitial inflammatory infiltrates mainly represented by macrophages.

Data from non-orthologous PKD animal models revealed that cyst formation precedes the detection of interstitial macrophage accumulation (92). A recent study is in line with such findings, indicating a role for MIF (macrophage migration inhibitory factor) in cyst growth and suggesting a non-initial, secondary role for macrophages in this process (93). The depletion of macrophages in a *Pkd1*-targeted PKD mouse, on the other hand, led to a milder cystic phenotype and a better renal function, suggesting a macrophage-dependent effect on cyst expansion (94). In this case, and in a *Pkd2*-targeted mouse model, the alternative M2-macrophage activation was found to be predominant. It is interesting to note that this pathway is generally related to a regenerative profile, differing from the oxidative and pro-apoptotic features of the classical M1-macrophage activation. In ADPKD, however, the proliferative, remodeling and pro-fibrotic effects of M2 activation are likely to play a significant role in cyst growth, renal fibrosis and renal function decline.

STAT3 is a likely contributor to establish and keep the PKD inflammatory environment. This pathway is activated in cyst-lining cells and is known to activate the transcription of cytokines and growth factors in tumoral cells. These mediators, in turn, are capable of activating STAT3 in associated M2-macrophages, which may induce a feed-forward loop between such cells.

TNF- α was shown to misposition PC2, preventing its expression in the PAC (95). Interestingly, this cytokine is capable of inducing the formation of cystic structures in cell cultures, an effect intensified in *Pkd2*^{+/-} cells. These findings suggest, therefore, a role for inflammation in cystogenesis.

Cell matrix alterations

Confocal microscopy and immunoprecipitation studies support a role for PC1 in cellular structures that mediate cell-matrix adhesion and components of various cell junctional complexes. ADPKD renal epithelial cells showed increased adhesion to type I collagen and express high levels of $\alpha 2\beta 1$ -integrin with overlapping colocalization with PC1 and focal adhesion proteins, as vinculin and paxillin (96). PC1 was also found in MDCK cell desmosomes, a cell structure specialized for cell-cell adhesion (97). In addition, yeast

two-hybrid experiments revealed intermediate filament protein vimentin as a strong PC1 interacting partner, as well as cytokeratins K8 and K18 and desmin (98). It must be noted that the PC1 lectin domain can bind to many extracellular matrix proteins, such as collagen type I, II and IV, a process that is amplified in the presence of calcium (99). The LRR domain appears to modulate PC1 binding to collagen I, fibronectin, laminin, and cyst fluid-derived laminin fragments (100). The observation that LRR induces reduction in cell proliferation suggests that this domain acts as a mediator of interactions between PC1 and extracellular matrix. In normal human fetal collecting tubules, immunocytochemistry and immunoprecipitation analyses showed that PC1 associates with focal adhesion proteins such as talin, vinculin, p130Cas, FAK, α -actinin, paxillin and pp60c-src (101). PC2 intracytosolic domain, in turn, was found in association with CD2AP, a protein adapter that regulates the assembly of focal adhesion complexes (102). Altogether, these studies place PC1 and PC2 dysfunction within the cell-matrix abnormalities observed in ADPKD.

Conclusion

The scientific information accumulated in the last two decades allowed expedited progress in the comprehension of ADPKD pathogenesis. Biochemical and molecular biology advances have provided key information to the elucidation of mechanisms involved in cyst development and disease progression. In addition to the basic and structural concepts presented and discussed in this chapter, the characterization of other cellular and systemic mechanisms has also contributed to understand fundamental features of this disorder, such as the process of renal function decline and its extra-renal manifestations. While a number of questions remain open in this research field, the fast growing knowledge brings promising perspectives for the understanding of ADPKD and additional therapeutic options.

Conflict of interest

The authors declare that they have no conflict of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 8

The Role of Calcium and Cyclic AMP in PKD

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Abstract

Cyclic AMP (cAMP)-driven mechanisms are central to the pathogenesis of polycystic kidney disease (PKD). Cyclic AMP stimulates both fluid secretion and cell proliferation, making abnormal cAMP-regulated pathways key targets for PKD therapy. The success of vasopressin receptor blockade in lowering cAMP levels and ameliorating disease in murine models of PKD and in a recent clinical trial, argues that cAMP-regulated mechanisms are fundamental to cyst formation and disease progression. This chapter focuses on why cAMP is important to the disease process, and how the primary abnormality in PKD is the abnormal response of cells to cAMP rather than high levels of cAMP *per se*. This abnormal

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cAMP response is a consequence of the calcium environment being disrupted in PKD from loss of polycystin function. We have identified signaling mechanisms by which decreased intracellular calcium levels can transform normal cells into PKD-like cells. By treating normal renal epithelial cells with calcium channel blockers it has been possible to de-repress B-Raf, allowing its activation by cAMP and subsequent MEK/ERK activation to stimulate cell proliferation. Autosomal dominant PKD (ADPKD) cells can also be switched back to a normal phenotype by raising intracellular calcium. The abnormal response to cAMP is made worse by mechanisms that further raise intracellular cAMP, causing cAMP to stimulate cyst-filling fluid secretion in a cystic fibrosis transmembrane conductance regulator (CFTR)-dependent fashion. The abnormal PKD-like phenotype is likely a result of misregulated gene expression as well as disruption of a number of signaling pathways and altered cell cycle control, all resulting in a change in the phenotypic state. It is hypothesized that disruption of the calcium/calcineurin/nuclear factor of activated T-cells (NFAT) pathway would contribute to this phenotypic change by altering gene expression, and activating and upregulating CDK4 causing loss of cell cycle control, events that would cause cyst initiation, and that would promote cyst growth and enlargement.

Key words: B-Raf; Calcineurin; CDK4; ERK; NFAT

Introduction

Polycystic kidney disease (PKD) is characterized by the abnormal growth of epithelial-lined cysts from the nephrons and collecting ducts of affected kidneys (1-3). PKD is associated with dramatic increases in kidney size, starting before birth, which results from the unrelenting growth of thousands of fluid-filled cysts, many undergoing massive enlargement. As cysts grow, they compress neighboring tubules and capillary circulation, causing functional nephron loss and promoting the development of fibrosis, destroying the surrounding renal parenchyma and interstitium (4, 5).

PKD is inherited as either an autosomal dominant condition (ADPKD) or an autosomal recessive condition (ARPKD). ADPKD is common, with a frequency of 1 in 400-1000 individuals, and results in 7-10% of all end-stage kidney disease (6). ARPKD is much less prevalent but has many features in common with ADPKD (7). Renal failure can occur in newborns or early childhood in the case of ARPKD or later in adulthood in the case of ADPKD. Mutations in the PKD1 gene or PKD2 gene are responsible for ADPKD and mutations in the PKHD1 gene are responsible for ARPKD. The products of these genes, polycystin-1 (PC1), polycystin-2 (PC2), and fibrocystin/polyductin are membrane proteins that are thought to regulate intracellular calcium in response to external stimuli (8, 9). PC2

is a transient receptor potential channel subunit (TRPP2) that forms complexes with PC1 and fibrocystin/polyductin, although the relevant cellular locations of these proteins and their specific functions at these sites are still being investigated.

Cyst formation occurs with loss of PKD gene function and a subsequent disruption in calcium homeostasis (10). In ADPKD, most cells appear to function normally in the heterozygous state and cysts form only sporadically throughout the kidney (Figure 1). As such, it is likely that there is a second initiating event – either a second somatic mutation or threshold event causing haploinsufficiency. As ADPKD cysts grow in size, they often remodel and pinch off from the tubule, and become isolated, self-contained structures (Figure 1, Right). These cysts continue to enlarge over years through a slow proliferative process, and they fill as they enlarge by secretion of fluid into the cyst lumen.

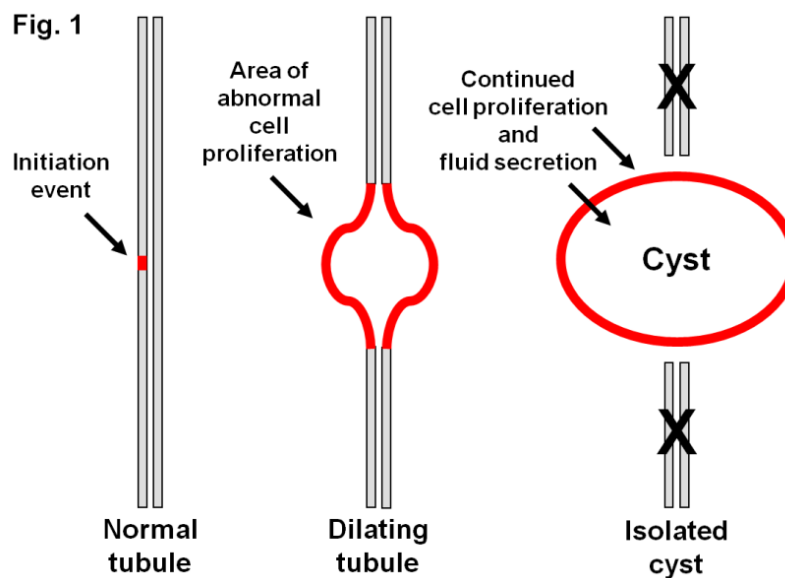


Figure 1. The process of cyst formation in autosomal dominant polycystic kidney disease. Each cyst is thought to initiate from a single cell, as the result of either a second somatic mutation or from a haploinsufficiency or threshold effect. Once that has occurred, it is thought that the abnormally proliferating cells, being incapable of forming a normal tubule, would cause a small region of the tubule to begin to dilate. As the cyst grows in size, it would become isolated by pinching off from the nephron. The cyst would then continue to enlarge over decades, through continued abnormal cell proliferation, and it would fill as it enlarges through a process of cystic fibrosis transmembrane conductance regulator -dependent fluid secretion. Cyclic AMP is important in driving both cell proliferation and fluid secretion.

In ARPKD, every tubule cell is homozygous for the PKHD1 mutation and thus is poised to initiate cell proliferation and cyst formation. It appears that large groups of cells or whole nephron segments lose their normal tubule morphology causing tubule dilatation along the axis of the nephron, in contrast to the focal cyst formation seen in ADPKD that is thought to arise initially from the transformation of a single cell. These cyst-like tubule dilatations in ARPKD may expand initially through glomerular filtration, although it is likely that they, too, depend on net fluid secretion as they enlarge.

However, in both ADPKD and ARPKD, loss of gene function alone is not sufficient to trigger abnormal cell proliferation. There is also an important role for cAMP. This chapter will examine the primary mechanisms in the cyst forming process: how normal cells become abnormal through loss of calcium homeostasis and cyclic AMP-driven cell proliferation and fluid secretion, mechanisms that are fundamental to cyst development and the progression of PKD.

A brief history of cAMP in cystic disease

Early studies carried out in cell culture demonstrated that cAMP is the key driver of cyst growth and expansion (11-20). Some of the earliest research recognized that ADPKD cysts enlarge in association with an accumulation of fluid within cysts as they form by the abnormal proliferation of tubule epithelial cells. In the initial stages of the disease (Figure 1), cysts appear as isolated structures throughout the kidney. At end-stage, polycystic kidneys are typically very large and packed full of fluid-filled cysts of various sizes. Cyclic AMP has been implicated in all aspects of the disease, from initiation, to progression, to end-stage (21).

There is no question that the growth and expansion of renal cysts in ADPKD is driven by cell proliferation. This idea came from early studies that showed the abnormal expression of genes involved in regulation of cell proliferation, including in particular, the oncogene *c-myc* (22-25). Renal cysts have been described as benign neoplastic growths, which unlike typical tumors are filled with fluid rather than being solid (26, 27).

Cyclic AMP was implicated in the growth of renal cysts using cell culture systems, with the demonstration that cAMP accelerates the enlargement of microcysts derived from MDCK and ADPKD cyst-lining epithelial cells growing in three-dimensional (3D) collagen gels (12, 14). These studies demonstrated increased microcyst growth, increased cell proliferation, and stimulation of transepithelial fluid secretion by prostaglandin E1 (PGE1), arginine vasopressin (AVP), cholera toxin, forskolin, 8-Br-cyclic AMP, and the phosphodie-

sterase inhibitor 1-methyl-3-isobutylxanthine (IBMX), in both established renal cell lines and primary cultures of normal human kidney and human cystic cells (11-20). All of these treatments would be expected to raise intracellular cAMP.

These studies further showed that fluid secretion by these cells is dependent on cAMP-mediated chloride secretion (18-21, 28, 29). As would be expected, it was found that in the absence of cAMP-based secretagogues, fluid was measurably *absorbed* from the microcyst cavity made up of ADPKD cells in collagen matrix (28). In contrast, 8-Br-cAMP plus IBMX induced a *reversal* in the net transport of fluid causing *secretion* into the lumen of the microcysts (28). Cellular chloride was also monitored, and changes in short circuit current (ISC) induced by forskolin in monolayers in the presence and absence of external chloride showed that cultured ADPKD cells can transport fluid in either direction, and that cAMP stimulates secretion dependent on the presence of chloride (28). Importantly, it has been demonstrated that CFTR-dependent chloride secretion drives net fluid secretion by ADPKD cyst epithelial cells (20, 29-31). Thus, cyst-filling fluid secretion is driven by cAMP.

The importance of cAMP and chloride secretion was further demonstrated in metanephric organ culture using embryonic kidneys from Pkd1^{-/-} mice (32). These kidneys thrive in culture over a 4-5 day period and can be treated with various agonists and inhibitors to determine their effects on the formation of cyst-like tubule dilations. As was shown in Magenheimer et al. (32), embryonic kidneys from Pkd1^{-/-} mice responded to the addition of 8-Br-cAMP to the culture medium by forming tubule dilatations that grew in size over several days. These dilatations were reduced by treatment with a protein kinase A (PKA) inhibitor, and were completely eliminated by genetic deletion of the CFTR gene, supporting the essential role of CFTR-dependent chloride secretion in this model system.

In summary, these studies identified that there are two essential cAMP-dependent components to cyst growth – cell proliferation and fluid secretion. Cyst growth cannot occur without both processes (see Figure 1). Cell proliferation is necessary to start the process, dilate the tubule, and enlarge the cyst from microscopic size to macroscopic. However, cell proliferation alone would not produce a cyst – only a relatively small solid clump of cells or adenoma, which would be much more benign than a cyst. Fluid secretion is also required, to fill the dilating structure and to enable it to swell to its enormous dimensions. However, fluid secretion alone would not produce a cyst. Without the formation of an actual enclosed cyst, increased secretion would only drain more fluid down the nephron. Thus, cAMP was shown to have two vital and essential roles in cyst formation, growth, and expansion, to stimulate both cell proliferation and cyst-filling fluid secretion.

High cAMP levels are associated with PKD

Yamaguchi et al. (33) found abnormally increased levels of cAMP in the kidneys and urine of homozygous pcy/pcy mice, which have a slowly progressive form of nephronophthisis (NPHP3). The cyst fluid from these mice and also from humans with ADPKD (34, 35) contained a lipid compound, later identified as forskolin (36), that stimulated both cAMP accumulation and cell proliferation of MDCK monolayers and increased transepithelial fluid secretion by these cells. Other studies have also shown that PKD kidneys have higher than normal levels of cAMP. Gattone et al. (37) showed higher renal cAMP in both pcy/pcy mice and PCK (PKHD1) rats, late in disease progression. Cyclic AMP levels were also measured in the kidneys of juvenile cystic kidney (jck/jck) mice, which have a mutation in the Nek8 gene, during early cyst formation at 26 postnatal days and later at 50 days (38). While there were no significant increases in the early phase of disease, there was very significant cAMP upregulation in the jck/jck cystic samples late in disease progression at 50 days. Large increases in cAMP were also seen in the urine of male Han:SPRD Cy/+ rats (39), and in a Pkd1 conditional null model in advanced-stage disease (40). As such, the results are consistent – that cAMP levels increase significantly in parallel with the degree of cystic disease.

What is the cause of the high cAMP?

Several potential causes for the increases in cAMP in PKD have been proposed. Gattone et al. showed that vasopressin receptor (V2R) mRNA was increased dramatically in early-stage postnatal cpk/cpk disease (41). Activation of V2R stimulates an increase in cAMP. This observation served as the basis for successful attempts to decrease cAMP and slow the development of cystic disease using the V2R antagonist OPC31260 in the cpk/cpk mouse and in later experiments using another OPC V2R antagonist Tolvaptan in other cell culture and animal models of PKD and in clinical trials (42-44). Consistent with these experiments, Wang et al. (45) showed that cyst formation could be effectively inhibited in PCK (PKHD1^{-/-}) ARPKD rats by crossing them with AVP deficient (AVP^{-/-}) Brattleboro rats, and that treatment of these rats with the AVP analog desmopressin (DDAVP) initiated cyst growth, clearly demonstrating the requirement for cAMP as an essential cyst-promoting factor in this genetically cystic model. The successful use of V2R blockade to lower cAMP and significantly slow or prevent cyst growth confirms the central importance of cAMP-driven mechanisms in PKD and is consistent with the major site of cyst formation being the collecting duct. However, the fact that cysts also form in other tubule segments, albeit with less overall impact, suggests that there are other mechanisms, as well, for increasing cAMP in addition to V2R stimulation.

Because of the calcium-impaired environment in PKD mutant cells, it has been suggested that calcium-regulated adenylate cyclases (ACs) and calcium-regulated phosphodiesterases (PDEs) may contribute to increasing cAMP. Pinto et al. (46) have shown that there is increased expression of the calcium-inhibited ACs 5 and 6 in ADPKD cells (but also an abnormally increased dependence on the calcium-stimulated AC3). Increased levels of ACs 5 and 6 and loss of their calcium-inhibition would be expected to generate increased cAMP. Indeed, this was supported by studies in which collecting duct conditional knockout of the AC6 gene ameliorated collecting duct-specific cystic disease (47). There was little or no impact of targeted AC6 knockout on overall kidney or urinary cAMP, indicating that decreasing cAMP specifically in the cyst cells *per se* was sufficient to reduce cyst growth. Recently, Pinto et al. (48) showed evidence for compartmentalized phosphodiesterase isoform regulation, where PDE4 appears to have a global role in regulating cAMP and cAMP-dependent fluid secretion, whereas the calcium-dependent PDE1 has a major role in regulating cAMP-dependent cell proliferation. Chebib et al. (49) have proposed an interesting hypothetical model in which increased cAMP in PKD cells results from a combination of events that ultimately causes the dysregulation of cAMP, including increased calcium-inhibited AC activity, decreased calcium-activated phosphodiesterase activity, and decreased calcium-dependent ATP release leading to decreased purinergic Gi-signaling that would normally limit vasopressin-dependent cAMP production. The result of this combination of events would be spiraling increases in cAMP in response to normal levels of vasopressin (49).

It is also quite possible that other mechanisms play a role in increasing cAMP. Of note is the observation that cyst fluid was found to contain a cyst-activating factor that promoted both cell proliferation and fluid secretion (33-35). When this factor was purified and identified by mass spectrometry, it was found to be a forskolin-like molecule (36). If it can be shown that forskolin, ordinarily thought to be specific to plants, is synthesized by animal cells, and particularly cyst-lining epithelial cells, this would provide a new mechanism for non-receptor-mediated upregulation of cAMP by direct stimulation of AC.

Pharmacological activation of the somatostatin receptor with somatostatin analogs has been used in clinical trials for ADPKD (50-52). These compounds activate Gi signaling, which down-regulates AC activity and lowers cAMP, indicating that Gi signaling has a significant impact on cAMP levels in PKD kidneys. Thus, since this is possible, it may also be the case that decreased Gi signaling by loss of PC1 activation of heterotrimeric G-protein signaling (53, 54) could lead to increased AC activity and higher cAMP. Finally, as an additional mechanism, GSK3 β has been shown to upregulate vasopressin-induced cAMP, promoting cyst growth in PKD through a positive feed-forward mechanism (55, 56). Thus, abnormal GSK3 β activation in PKD (57) could contribute to high levels of cAMP in the collecting duct.

Thus, there are many possible mechanisms for increasing cAMP, and for maintaining high levels in the collecting ducts and elsewhere in the kidney. These observations support the view that cAMP-driven mechanisms are important in PKD. However, it should be recognized that cAMP levels are often normal early in the disease process. Furthermore, cAMP itself is not cystogenic in wild-type animals (45), and there is no evidence that elevated cAMP alone can convert a normal cell to a cystic cell. Therefore, while cAMP is an essential (or critical) cyst-promoting factor, abnormally elevated cAMP alone cannot be the sole cyst-forming determinant.

Why high cAMP alone is not the main disease culprit?

Hanaoka et al. (31) and Yamaguchi et al. (58) demonstrated that cAMP directly stimulates ADPKD cell proliferation, and Yamaguchi et al. (58) went on to demonstrate that this involved activation of the mitogen-activated protein kinase (MAPK) pathway (Figure 2). In these studies, primary epithelial cells from cysts of ADPKD kidneys and from normal human kidney cortex were studied in culture. The effects of agonists and inhibitors on cell proliferation and activation of the extracellular signal-regulated kinase (ERK1/2) pathway were determined. Direct stimulation with 8-Br-cAMP was seen to increase the proliferation of the ADPKD cells (58), and this proliferation was inhibited by PKA inhibitors. The cAMP-generating agonists AVP, DDAVP, secretin, vasoactive intestinal polypeptide (VIP), forskolin, and prostaglandin E2 (PGE2) also stimulated proliferation. The MEK (mitogen/extracellular signal-regulated kinase) inhibitor PD98059 effectively inhibited ADPKD cell proliferation in response to cAMP agonists, whereas genistein, a receptor tyrosine kinase inhibitor, did not block cAMP-dependent proliferation (58). Importantly, cells from normal human kidneys responded in an opposite fashion to cAMP agonists by showing decreased cell proliferation (Figure 2, Left vs. Right) (31, 58).

Thus, it was evident from these studies that cAMP agonists stimulate the proliferation of ADPKD but not normal epithelial cells through PKA activation of the ERK pathway through a B-Raf dependent mechanism and that cAMP may play a critical role in ADPKD by driving cell proliferation (58, 59). Indeed, it appeared that cAMP could stimulate the proliferation specifically of ADPKD cyst cells, while not affecting the surrounding normal renal tubule cells, resulting in focal cyst growth. These experiments showed that it was not the level of cAMP, but the *response* to cAMP that characterized the cyst-promoting activity of cAMP, and this being the case that higher levels of cAMP would certainly exacerbate this pathogenic process.

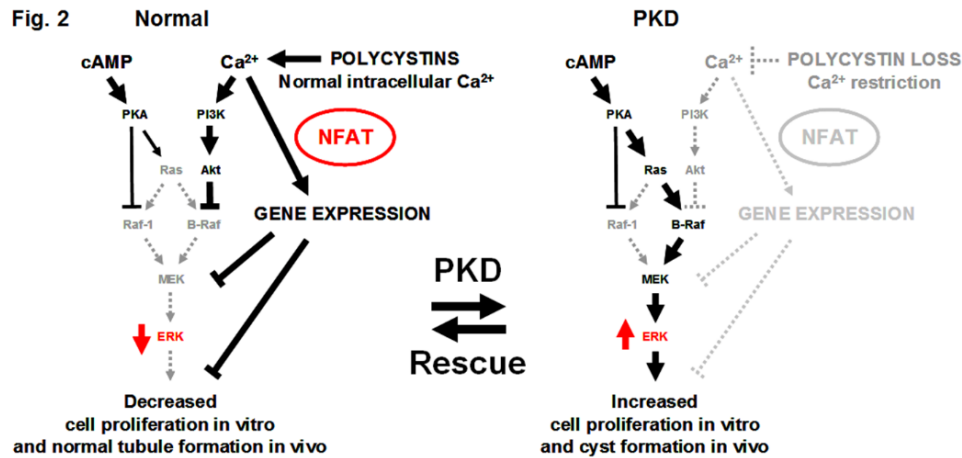


Figure 2. Cyclic AMP signaling in normal and PKD cells. Signaling pathways are shown from cAMP and calcium to extracellular signal regulated protein kinase (ERK) and cell proliferation. Left. Normal signaling pathways. Right. Signaling pathways in PKD due to loss of polycystins and/or disrupted intracellular calcium and NFAT-regulated gene expression. Solid lines = active pathways. Dotted lines = diminished pathways. PKD cells, shown on the right, differ phenotypically from normal cells (on the left) in part because loss of polycystin signaling in ADPKD disrupts the PI3K/Akt pathway and NFAT signaling, converting the cellular response to cAMP from anti-mitogenic to mitogenic. The same effect is seen by stably transfecting normal M-1 cells with a dominant negative polycystin-1 C-tail construct. ADPKD cells or PKD-like cells can be rescued by treatment with a calcium ionophore. Adapted from Figure 11 in Yamaguchi et al. (61).

The preceding experiments were instrumental in showing that ADPKD cyst-derived cells have a demonstrable phenotypic abnormality – their abnormal response to cAMP made worse by high levels of the cyclic nucleotide. However, it was not clear from these experiments, which used human ADPKD cystic cells cultured from end-stage kidneys, whether this abnormal responsiveness to cAMP was due only to PKD mutation and loss of polycystin function, or to other secondary events resulting from decades of accumulated genomic mutations and/or epigenetic changes. In principle, a second-hit initiation event could set off a cascade of secondary mutagenic events whose accumulated effects might lead to an abnormal, transformed cellular phenotype manifested by an altered sensitivity to cAMP.

Sutters et al. (60) answered this question by the development of a cell line that stably expressed a dominant-negative Pkd1 construct in immortalized M-1 renal collecting duct

cells. Cells expressing the mutant PC1 C-tail construct (Clone 20) were dexamethasone inducible and could be directly compared to cells transformed with a control construct (Clone 17) that while integrated did not express the mutant PC1 C-tail. Thus, comparable cell lines, induced to overexpress a mutant PC1 construct or no PC1 construct, could be grown in culture and their responses to cAMP examined. In proliferation assays, the mutant PC1 clone grew somewhat faster than the control clone, due presumably to low levels of endogenous cAMP, and when exogenous cAMP was added to the culture medium, the differences in their growth rates were pronounced, with Clone 20 being stimulated to proliferate, and Clone 17 being inhibited. The behavior of other clones was also examined, and in every case, cells carrying these dominant-negative PC1 constructs responded like the ADPKD cyst cells while the cells carrying control constructs behaved like normal renal epithelial cells (and the parental wild-type M-1 cells). These mutant PC1 clones responded in the same way to 8-Br-cAMP and other cAMP agonists, including forskolin, AVP, DDAVP, VIP, secretin, and PGE₂, and this response could be blocked by PKA inhibitors but not by the tyrosine kinase inhibitor, genistein. The fact that the MEK inhibitor PD98059 was effective in blocking proliferation suggested that activation of the MAPK pathway was necessary for the cAMP stimulated cell proliferation. As such, these experiments showed that cells could be made to change their phenotype by overexpressing the PC1 C-tail to mimic the cAMP-responsive cyst-forming phenotype of ADPKD cells.

Expression of a dominant-negative PC1 construct changed the phenotype of these cells from one that was inhibited by cAMP to one that was stimulated by cAMP. The mutant PC1 C-tail construct did not act simply to alter the degree of cAMP-responsiveness of these cells, but acted by switching cells from one state to another. This phenotypic change was not caused by a changing level of cAMP, since both the control cell line and the mutant cell line increased their cAMP levels when treated with cAMP agonists. As such, it is not the high levels of cAMP but the opposite *responses* to cAMP that *distinguish and define* the normal and PKD phenotypes.

What is the primary abnormality?

While there is general consensus that the PKD genes regulate intracellular calcium, there is uncertainty as to where and under what conditions the gene products function, and what the calcium signal actually does to the cell. Despite this critical lack of knowledge, we reasoned that if the polycystin proteins regulate calcium, and since there is loss of the polycystins in PKD, then perhaps a decrease in intracellular calcium is the primary causative abnormality in PKD, converting the cell proliferation phenotype from cAMP-inhibited to cAMP-stimulated. If so, it might be possible to model the PKD phenotype in

cell culture by artificially reducing the levels of intracellular calcium in genetically normal cells (see Figure 2, Left vs. Right).

This experiment was accomplished by Yamaguchi et al. (61) using primary normal human kidney cells and immortalized M-1 cells treated in various ways to lower intracellular calcium, and then assayed for cell proliferation and ERK activation. Both M-1 cells and normal HKC (human kidney cortex) cells showed decreased cell proliferation when treated with 8-Br-cAMP, but increased cell proliferation when pre-treated with the calcium channel blockers, Nifedipine, Gadolinium, or Verapamil, prior to treatment with 8-Br-cAMP. Lowering free extracellular calcium with EGTA also resulted in cAMP stimulation. Thus, normal cells could be made to switch their phenotype to PKD-like cells simply by lowering intracellular calcium. Of interest was the observation that the above-mentioned stably transfected M-1 Clone 20 cells, which behaved like PKD cells (60, 62), could be normalized with respect to their cAMP-responsiveness by treatment with the calcium ionophore A23187, further validating the PC1-mutant Clone 20 cells as a faithful model of ADPKD cells and underscoring the importance and relevance of calcium in controlling the cAMP-response.

Cyclic AMP stimulation of the calcium-restricted HKC and M-1 cells was further analyzed and found to function through activation of the Ras/MAPK pathway in a PKA and Src-dependent fashion (61). This analysis revealed how the phenotypic switch is controlled by calcium. Normally growing cells in culture, in response to serum and other autocrine or paracrine growth factors in the medium, have an active Ras/MAPK pathway leading to ERK activation and cell proliferation. The absence of cAMP allows signal transduction through the MAP3K, Raf-1. If these cells are treated with 8-Br-cAMP or a cAMP agonist (Figure 2, Left), the growth factor signal will be inhibited by PKA phosphorylation and inactivation of Raf-1, and there will be decreased ERK phosphorylation and decreased cell proliferation. Under conditions of calcium restriction, as in PKD (Figure 2, Right), there is decreased intracellular calcium, which inhibits calcium-dependent PI3K and deactivates its downstream target Akt. These events result in the loss of an inhibitory Akt phosphorylation of B-Raf, causing its de-repression, allowing the cAMP signal to bypass inactive Raf-1 to upregulate ERK phosphorylation and increase cell proliferation. In other words, the ADPKD state is caused by cAMP-activated mitogenic stimulation in a cellular context made permissive by decreased calcium, as can be modeled simply by lowering intracellular calcium levels and then treating cells with cAMP agonists. These studies are the first to describe a phenotypic switch of this nature, in which a genetically normal cell is transfigured to adopt an abnormal phenotype simply by lowering tonic intracellular calcium levels.

The primary ADPKD cells described above (58) have an approximately 20 nM lower intracellular calcium concentration than their normal counterparts (63), which can explain their mitogenic response to cAMP. Importantly, these primary human ADPKD cells and primary human ARPKD cells (63) can be switched back to normal simply by raising intracellular calcium using the calcium channel activator Bay K8644, or the calcium ionophore A23187 (Figure 2, Rescue). Of significance is that these experiments take a genetically-programmed cell with an abnormal phenotype and reestablish its normal behavior with increased cellular calcium, which overrides the genetic damage (63).

Animal studies have supported and extended these observations. Using the Cy/+ Han:SPRD rat model of dominant PKD, it was possible to show that treatment of cystic rats with Verapamil dramatically exacerbated cystic disease, as determined by kidney weight and cystic index (39). A dose of Verapamil was used sufficient to normalize blood pressure increases in these animals. Increased cyst growth was accompanied by increased cell proliferation, and phosphorylation and activation of ERK, and was presumed to be in response to the endogenous cAMP. These responses were not seen in wild-type kidneys. Thus, in a non-orthologous model in which cyst formation occurs predominantly in proximal tubules, calcium appears to play a role in the disease state, consistent with the cell culture models. Experiments using the calcimimetic R-568 to raise intracellular calcium in pcy/pcy mice reduced cyst enlargement and renal fibrosis (64). Interestingly, the Chinese herbal active ingredient Triptolide, which acts by increasing intracellular calcium, has been shown to slow cell proliferation and ameliorate cystogenesis in fetal mouse kidneys when administered during pregnancy, and in a postnatal conditional Pkd1 mouse model (65, 66). Calcium has also been shown to be important in 3D microcyst cultures in which knockdown of PC2 or the inositol 1,4,5-trisphosphate receptor supported cyst growth (67); and in metanephric organ culture in which cystic dilations were reduced by treatment with a calcium ionophore (68).

In the experiments by Yamaguchi et al. referred to earlier (61), it was evident that lowering intracellular calcium using a variety of approaches was effective in converting normal cells to PKD-like cells (see Figure 2). However, in these experiments, it was noted that calcium restriction required hours-long treatments. With EGTA, a minimum of 3 hours was required, and with Verapamil, a minimum of 5-8 hours was required, depending on the dose, with 16 hours treatment being most effective. It was also demonstrated that once the phenotypic switch was established with an 8-hour Verapamil treatment, it remained in place following up to a 12-hour washout period in which cells were no longer exposed to Verapamil. These observations were inconsistent with a simple model in which the sole effect of decreased calcium is inhibition of the PI3K/Akt signaling pathway since Verapamil treatment is likely to affect intracellular calcium within minutes. A requirement

for hours-long treatments is suggestive of a need to alter gene expression through changes in gene transcription, protein translation, and/or turnover of existing mRNAs and proteins to bring about a change in the differentiated state of the cell.

In thinking about a role for the protein products of the PKD genes in regulating gene expression, the following should be considered. As mentioned earlier, PC1 and PC2 are thought to regulate intracellular calcium, probably in response to a ligand-mediated event or a mechanosensory stimulus (69). While PC2 is known to be a calcium-regulated cation channel (70), PC1 has also been shown to be capable of elevating intracellular calcium through a heterotrimeric G protein-coupled mechanism (53, 54, 71). Thus, it is likely that PC1 and PC2 proteins function together as part of a multi-protein membrane complex to regulate a number of calcium-dependent signaling pathways that ultimately regulate gene expression. Importantly, among the downstream targets regulated by polycystin-1 is the calcium-dependent phosphatase calcineurin and its immediate substrate, the transcription factor NFAT (Figure 2, NFAT) (71).

What causes the phenotypic switch? An hypothesis

We suggest that calcium-restriction does two things to renal epithelial cells. In the short term, it inhibits PI3K/Akt thus de-repressing B-Raf, but then, in the long term, it alters calcium-dependent gene expression. It is proposed that both are needed to bring about the switch to the PKD state. As shown in Figure 2, it is suggested that the calcium-dependent transcription factor NFAT is involved in mediating this phenotypic switch. The significance of this idea is that polycystin-dependent calcium signaling, acting to regulate the phenotypic state, may protect cells from the effects of normal fluctuations of cAMP, or to increases in cAMP above normal levels. Loss of polycystin-regulated calcium signaling would render cells inappropriately vulnerable to this cAMP.

There are four calcium/calcineurin-regulated NFAT family members NFAT1 (p, c2), NFAT2 (c, c1), NFAT3 (c4), and NFAT4 (x, c3), all expressed in the kidney (72-79). The NFAT proteins are maintained in an inactive state in the cytosol as phosphoproteins (P-NFAT) (Figure 3). Various phosphatases and kinases regulate the nuclear (active) or cytoplasmic (inactive) localization of NFAT. Regulation of NFAT involves calcium-dependent activation of the serine threonine phosphatase, calcineurin (Caln, PP2B, Ppp3ca), which dephosphorylates NFAT resulting in its translocation to the nucleus (Figure. 3, Left). Once in the nucleus, NFAT can bind DNA elements in target promoters often in association with other co-induced nuclear proteins, such as AP-1, GATA, and NF- κ B, to regulate gene expression. The continuous maintenance of NFAT in the nucleus

requires sustained, oscillatory, calcium increases (80) to keep calcineurin in an activated form. This calcineurin-dependent signal is sensitive to inhibition by calcium channel blockers such as Verapamil or calcineurin inhibitors such as Cyclosporin A (CSA) or Tacrolimus (FK-506) (Figure 3, Right) (81-83). A number of protein kinases, in particular, GSK3 β , act to phosphorylate nuclear NFAT, driving it back to the cytoplasm (84). As such, there is a continuous, calcium-regulated 'push-pull' of phosphorylation-dephosphorylation affecting active nuclear NFAT levels.

The NFAT proteins are highly homologous and have partially overlapping functions. Interestingly, calcineurin A- α knockout results in impaired kidney growth, consistent with a role for NFAT in kidney development (85, 86). Additionally, calcineurin knockout alters trafficking of aquaporin-2 (AQP2) and causes diabetes insipidus (87), suggesting that NFATs are involved in adaptive responses in the kidney. Recent data have demonstrated that renal development is absolutely calcium-dependent (88) requiring the non-canonical calcium/NFAT Wnt signaling pathway (86). A role for NFAT in the kidney is consistent with the known nephrotoxicity of Cyclosporin A (89). Additionally, the Cox-2 gene is an NFAT target in the kidney (90), and disruption of the Cox-2 gene gives rise to cysts (91), suggesting that there may be a connection between NFAT and cystic disease through Cox-2.

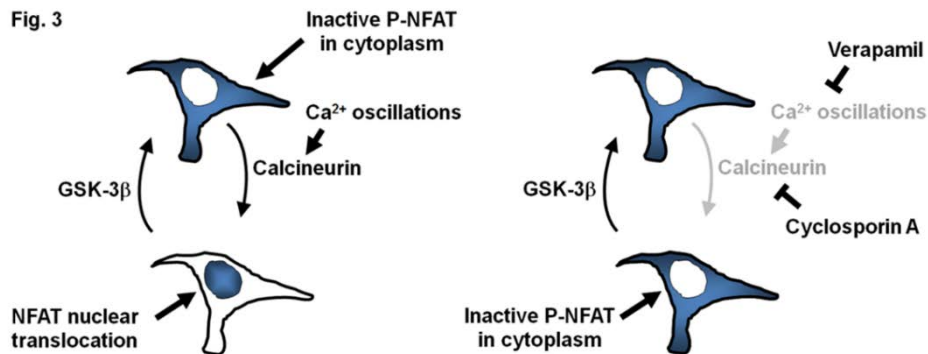


Figure 3. Mechanism of nuclear factor of activated T-cells (NFAT) regulation. Shown are cells containing cytosolic or nuclear NFAT (blue shading). (Left) The inactive hyperphosphorylated NFAT (P-NFAT) which resides in the cytosol, is dephosphorylated by calcineurin following activation by calcium oscillations, allowing NFAT to translocate to the nucleus where it activates or represses genes together with other transcription factors. NFAT is then re-phosphorylated by cellular kinases, including GSK3 β , returning it to the cytoplasm. (Right) NFAT activation can be inhibited by treating cells with calcium channel blockers, such as Verapamil, or with calcineurin inhibitors, such as Cyclosporin A. It is hypothesized that decreased intracellular calcium resulting from polycystin loss inhibits NFAT activation, contributing to the PKD phenotypic switch.

Importantly, the calcium/calcineurin/NFAT pathway also has a cell cycle role that may be directly relevant to PKD. The cyclin-dependent kinase CDK4 has been shown to be a key player in cell proliferation associated with PKD (92-96). CDK4 is under the regulation of cAMP (97) and other pathways relevant to PKD (94), but also calcineurin and NFAT through different mechanisms. NFAT transcriptionally down-regulates the CDK4 gene (98, 99). As such, decreased nuclear NFAT activity would be expected to increase CDK4 protein. For its activation, CDK4 requires phosphorylation of Threonine-172 (T172) by an ill-defined cyclin-activating kinase, which is regulated indirectly by cAMP (97). T172 phosphorylation is reversed by calcineurin phosphatase activity (100), causing its inactivation. Therefore, decreased calcium/calcineurin signaling in PKD would lead to increased CDK4 levels and activity, promoting cell cycle entry, a requirement for cyst growth and enlargement. As such, impaired calcium signaling in PKD could bring about a phenotypic switch at multiple levels, both upstream and downstream of ERK (Figure 2).

Conclusions

There is ample evidence demonstrating that PKD is associated with abnormally high levels of cAMP. However, it is also evident that the essential abnormality in PKD is not high cAMP *per se*, but the *response* of cells to cAMP. The basis for this abnormal response to cAMP is that calcium regulation is disrupted in PKD as a consequence of abnormal polycystin function, resulting in a number of signaling and gene expression changes that cause cells to respond abnormally to cAMP regardless of whether the levels are physiologically normal or elevated. One of the abnormal signaling events is the loss of calcium inhibition of B-Raf, which allows cAMP to activate B-Raf, MEK, and ERK. However, it is likely that additional abnormalities are required to transform the phenotype of a cell. It is hypothesized that one such abnormality results directly from decreased calcium, which would impair calcineurin function and decrease the activity of the NFAT transcription factor, affecting the differentiated state of these cells and their cell cycle control, and leading to the transformation of normal tubular epithelial cells to cystic epithelial cells that would continue to divide unchecked as they grow into massive fluid-filled cysts.

Conflict of interest

The author declares that he has no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 9

Apoptosis in Polycystic Kidney Disease: From Pathogenesis to Treatment

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Abstract

Apoptosis plays an important role in many developmental processes and contributes to cell and tissue homeostasis. Induction of apoptosis can involve the "intrinsic pathway", which is activated by diverse stress signals, and the "extrinsic pathway", which is activated by proapoptotic receptor signals at the cell surface. Excessive or aberrant apoptosis is a crucial factor in many human disorders, including polycystic kidney disease (PKD). Renal cyst formation is caused by dysregulation of cell proliferation, involving diverse and poorly understood molecular mechanisms. Elevated apoptosis of tubular epithelial cells has been

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described in autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD), as well as in animal models of PKD. It has been suggested that the dysregulation of apoptosis contributes to cystogenesis of PKD and is associated with the progressive loss of normal nephrons. Inhibition of apoptosis has been shown to delay renal cyst growth in some animal models of PKD. However, increased apoptosis is not a feature in cystic kidneys from *Pkd1* mutant mice and inducing apoptosis of the cystic epithelial cells by activation of intrinsic or extrinsic signaling pathways has been shown to slow disease progression with or without inhibition of proliferation. In this chapter, we discuss the positive and negative roles of apoptosis in PKD and the associated molecular mechanisms in regulating cystic renal epithelial cell apoptosis during cyst development.

Key words: Apoptosis; Caspases; Cystogenesis; Mitochondrial pathway; Polycystic kidney disease; Proliferation

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic kidney disease with the prevalence between 1:400 and 1:1000 worldwide and is characterized by the progressive development of renal cysts that replace normal kidney tissue, resulting in kidney expansion and the decline in renal function, often requiring dialysis or kidney transplantation (1). ADPKD is caused by the mutations in *PKD1* (85% of cases) or *PKD2* (15% of cases), which encode polycystin 1 (PC1) and polycystin 2 (PC2), respectively. PC1 and PC2 are large transmembrane proteins localized at primary cilia and plasma membranes, and regulate multiple intracellular signaling pathways (2).

Autosomal recessive polycystic kidney disease (ARPKD) is less common than ADPKD with a prevalence of 1:20,000 live births. ARPKD is caused by mutations in the *PKHD1* gene, which encodes fibrocystin, a large type I transmembrane protein localized to the apical membrane, the primary cilium/basal body and the mitotic spindle (3). Abnormalities in the renal epithelial cells, including dedifferentiation, dysregulated cell proliferation, abnormal fluid secretion, abnormalities of cell-matrix interactions, loss of planar cell polarity and abnormal ciliary function have been found to contribute to cystogenesis in genetic forms of PKD. Currently, the role of apoptosis in normal kidney development (4) and cyst growth (5) remain elusive. This chapter summarizes the current knowledge on the roles and mechanism of apoptosis in PKD and how components of apoptotic signaling can be used as therapeutic target in PKD.

Apoptosis

Apoptosis is a term that originates from the Greek word for “dropping off” and refers to the Autumnal falling of leaves from trees as described by Kerr et al. in 1972 (6). It is an orchestrated event in which cells are programmed to die upon receiving certain physiological and pathological stimuli (6), whereas not all cells will necessarily die in response to the same stimulus. As one of the most investigated processes in biological research, apoptosis occurs normally to maintain tissue homeostasis or as a defense when cells are damaged by disease or noxious agents (7). Abnormalities in apoptosis can be one of the significant features of diseases, including the insufficient apoptosis in cancers and excessive apoptosis in autoimmune and neurodegenerative diseases, and ischemia-associated injury (8).

Morphological and biochemical changes in apoptotic cells

Apoptosis is accomplished by a series of energy-requiring biochemical events which lead to the characterized morphologic changes. The classic morphological hallmarks of apoptosis during the early process, that is distinct from necrosis, include chromatin condensation, nuclear fragmentation, cellular volume reduction (pyknosis), and all of these morphological changes are enclosed within an intact plasma membrane. The morphological features of apoptosis during the late process include blebbing of plasma membrane, ultrastructural modification of cytoplasmic organelles including the swelling of mitochondria and loss of membrane integrity before the phagocytosis (8). During apoptosis, a process called “budding” occurs whereby extensive plasma membrane blebbing and separation of cell fragments into apoptotic bodies occur. The apoptotic bodies consist of cytoplasm with tightly packed organelles with or without nuclear fragments. They are subsequently phagocytosed by macrophages, parenchymal cells, or neoplastic cells and degraded within phagolysosomes (9-12).

The characterized biochemical events of apoptosis include cleavage and activation of caspases, breakdown of DNA and proteins, and modification of the membrane for phagocytic cell recognition (13). Caspases are a group of enzymes belonging to the cysteine protease family, and are widely expressed in an inactive proenzyme form in most cells. The “c” of “caspase” refers to a cysteine protease, while the “aspase” refers to the enzyme’s unique property cleave proteins at aspartic acid residues. Caspases have been broadly categorized into apoptotic caspases, including initiators (caspase-2,-8,-9,-10) and executioners (caspase-3,-6,-7) (14, 15), and inflammatory caspases (caspase -1,-4,-5,-11,-12) (14, 15). Once caspases are initially activated, initiator caspases cleave inactive forms of executing caspases, thereby activating them. Executing caspases cleave other vital cellular

proteins, break up the nuclear scaffold and cytoskeleton, and activate DNAase to degrade nuclear DNA, which triggers the morphological characteristics of the apoptotic process (16). Another feature of apoptosis is the “flip out” of phosphatidylserine from the inner layers to the outer layers of the cell membrane due to the loss of ATP. This allows the early recognition of dead cells by macrophages, resulting in phagocytosis without the release of pro-inflammatory cellular components (17). Apoptosis can occur without oligonucleosomal DNA fragmentation and can be caspase-independent (18), therefore we cannot always define apoptosis by using biochemical analyses of DNA fragmentation or caspase activation.

Methodologies and assays for detecting apoptosis

Apoptosis can be detected by multiple approaches since it occurs via a complex signaling cascade and has many specific features. Apoptotic assays can be classified into six groups (8), including: 1) cytomorphological alterations detected by light microscopy and electron microscopy, which is the gold standard for apoptosis identification; 2) DNA fragmentation detected by staining with a cell-permeable, DNA-binding fluorochrome in the terminal transferase-mediated dUTP nick end-labeling (TUNEL) assay (19); 3) detection of caspases, cleaved substrates, regulators and inhibitors such as cleaved caspase 3, and cleaved PARP by Western immunoblots or immunohistochemistry (20, 21); 4) membrane alterations detected by staining with FITC-labeled Annexin V that recognizes the phosphatidylserine (22); 5) detection of apoptosis in whole mounts by using dyes such as acridine orange (AO), Nile blue sulfate (NBS), and neutral red (NR) (23); and 6) mitochondrial assays to detect the cytochrome c release (24), the apoptotic or anti-apoptotic regulator proteins such as Bax, Bid and Bcl-2 (25), the mitochondrial membrane potential, calcium fluxes and mitochondrial redox status (26). Each assay has its advantages and disadvantages (27, 28). In addition, because many features of apoptosis and necrosis overlap, it is necessary to determine that apoptosis has occurred in cells, tissues or organs using two or more distinct assays based on the understanding of the principles of each methodology.

Mechanisms of apoptosis

Understanding the mechanisms of apoptosis helps in the development of drugs that target certain apoptotic genes or pathways. There are two commonly-described apoptotic pathways: the extrinsic or death receptor pathway and the intrinsic or mitochondrial pathway (Figure 1). Different caspases are activated in each pathway and molecules in one pathway can influence the other (29). There are two less well-known pathways, including the pathway that involves T-cell mediated cytotoxicity (30) and the intrinsic endoplasmic reticulum (ER) pathway (31), which will not be discussed in this chapter.

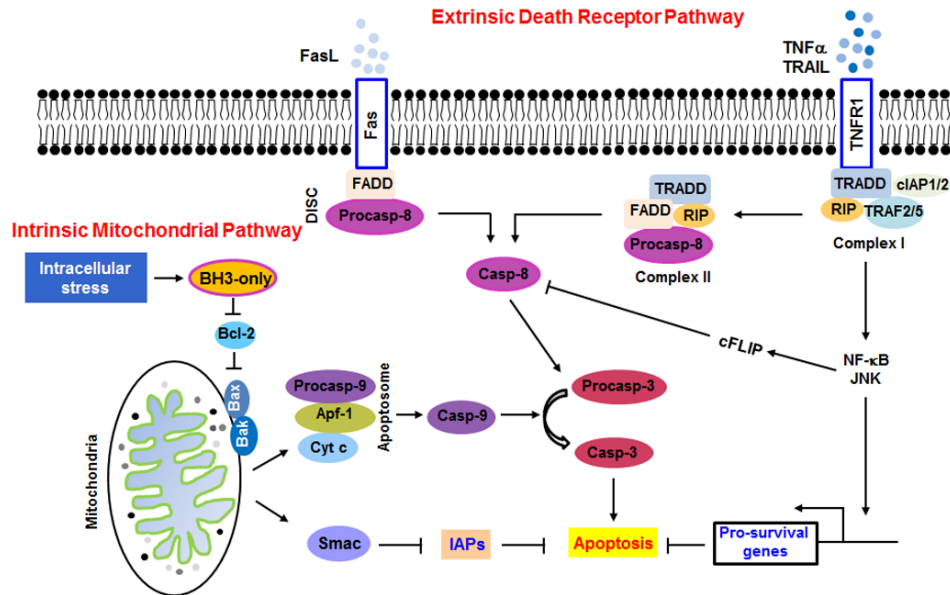


Figure 1. The intrinsic and extrinsic pathways of apoptosis. The two major apoptotic pathways are illustrated. The extrinsic death receptor pathway is activated by death receptor ligands, including FasL, TNF- α or TRAIL, etc. The binding of FasL to Fas initiates the recruitment of FADD and caspase-8 to form the DISC complex, which in turn activates caspase-8 and downstream executing caspases. The binding of TNF- α to TNFR1 initiates the recruitment of TRADD, RIP, TRAF2/5 and cIAP1/2 to form complex I, which activates NF- κ B and JNK pathways and increases the transcription of pro-survival genes. However, the modification of RIP or degradation of cIAP1/2 can lead to the disassociation of complex I. TRADD and RIP then associate with FADD and caspase-8 to form complex II, the so-called death complex. The intrinsic death receptor pathway is initiated by BH3-only protein under intracellular stress; BH3-only protein can inactivate Bcl-2 and prevent Bcl-2 from effectively neutralizing Bax and Bak, leading to activation of Bax and Bak. The activated Bax and Bak on the mitochondrial membrane result in the release of cytochrome c and Smac from mitochondria. Cytoplasmic cytochrome c associates with Apaf-1 and caspase-9 to form the apoptosome, which activates caspase-9 and downstream executing caspases. Smac can regulate apoptosis by inhibiting the inhibitor of apoptosis proteins (IAPs).

The extrinsic death receptor pathway

The extrinsic pathway is initiated by the binding of ligands to the transmembrane death receptors. Several death ligands and corresponding death receptors have been described, including Fas ligand and Fas receptor (FasL/FasR), tumor necrosis factor (TNF) and its

receptor 1 (TNF- α /TNFR1), Apo3L/DR3, Apo2L/DR4 and Apo2L/DR5 (8). As death receptors, members of TNF receptor superfamily share similar cysteine-rich extracellular domains and a cytoplasmic death domain (32). This death domain plays a critical role in transmitting the extracellular death signaling to the intracellular apoptotic machinery to elicit cell death through recruiting adaptor proteins, such as Fas-associated death domain (FADD) and TNF receptor associated death domain (TRADD). To date, the extrinsic pathways of apoptosis mediated by FasL/FasR and TNF- α /TNFR1 are the best-characterized. The binding of FasL to its FasR results in the recruitment of the adaptor protein FADD (33). FADD then associates with procaspase-8 to form the death-inducing signaling complex (DISC), which results in the activation of procaspase-8 (33, 34). Activated caspase-8 then triggers the execution phase of apoptosis including the activation of caspase 3 and downstream pathways. In contrast, the binding of TNF to TNFR results in the transient recruitment of TRADD, TNF receptor-associated factor 2 (TRAF2), TRAF5, cellular inhibitor of apoptosis 1 and 2 (cIAP1/2) and receptor interacting protein 1 (RIP1) to form pro-survival complex I (35, 36). Complex I can activate nuclear factor κ B (NF- κ B) and JNK pathways to regulate the expression of pro-survival genes, including the cellular FLICE-like inhibitory protein (cFLIP) (36, 37). However, TRADD and RIP1 can be disassociated from complex I once RIP1 is deubiquitinated under certain circumstances. RIP1 then associates with FADD and caspase-8 to form complex II, the so-called death complex, to trigger cell death (36). Death receptor mediated apoptosis can be inhibited by affecting the recruitment of caspase-8 to the DISC. cFLIP competitively displaces caspase-8 from the DISC due to the structure similarities with caspase-8 (38, 39). The cIAP1/2 also inhibits caspase-8 activation via suppressing TNF- α apoptotic signaling and inducing pro-survival signaling, such as the NF- κ B pathway (40).

The intrinsic mitochondrial pathway

The intrinsic pathway is initiated within the cell and involves the intracellular organelles mitochondria (41). The intrinsic pathway is regulated by the members of Bcl-2 family, which has been divided into three groups with different functions, including 1) antiapoptotic proteins (Bcl-2, Bcl-xL, etc.), 2) proapoptotic proteins (BAX, BAK, etc.) and 3) regulatory BH3-only proteins (BAD, BIK, BIM, etc.) (42, 43). The proapoptotic proteins are inhibited by the antiapoptotic proteins, while the BH3-only proteins can counteract the antiapoptotic proteins and thereby release the proapoptotic proteins to trigger the intrinsic apoptotic pathway upon the cytotoxic stimuli (42, 43). Upon the internal stimuli such as hypoxia, DNA damage and toxins, two proapoptotic BCL2 proteins, BAX and BAK, are activated by BH3-only proteins (44). Activated BAX and BAK oligomerize on the mitochondria and introduce pores into the mitochondrial surface, which results in the release of cytochrome c (45). Cytoplasmic cytochrome c associates with the caspase adaptor

molecule Apaf-1 and procaspase-9 to form the apoptosome, in which procaspase-9 is activated (45). The activation of caspase-9 then triggers the execution phase of apoptosis. The IAPs are also the negative regulators of the intrinsic apoptosis pathway by several mechanisms (46). In addition to cytochrome c, the BAX/BAK proteins can also induce the release of the second mitochondrial-derived activator of caspase (Smac)/direct IAP binding protein with low pI (DIABLO) from mitochondria, which disrupts the interaction of IAPs with caspase-3/9, and then promotes caspase activation (47, 48).

Apoptosis in polycystic kidney disease

Apoptosis was first described as a pathological feature of PKD by Woo in 1995 (49), in that apoptotic DNA fragmentation and nuclei were detected in cystic renal cells and tissues from human ADPKD and ARPKD, the congenital polycystic kidney (cpk) mouse model of ARPKD, and the pcy mouse model that is orthologous to adolescent nephronophthisis (49). Abnormal apoptosis observed in cyst lining epithelial cells and noncystic tubular epithelial cells, as well as in cells within glomeruli, has been suggested to contribute to cyst extension in human PKD (49, 50). This hypothesis has been supported by different studies, including those in ARPKD animal models and in 3D cultures with Pkd1 overexpression or knockdown (51, 52). Whether apoptosis is changed in Pkd1 and Pkd2 knockout animal models is uncertain. Recent studies have indicated that induction of cyst lining epithelial cell apoptosis may delay renal cyst growth in Pkd1 knockout mouse models (53-58). These studies suggest that the role of apoptosis in promotion or retardation of cyst growth in PKD could be disparate, depending on confounding factors such as animals compared to humans, early compared to late disease, tubules and interstitial cells compared to cyst-lining epithelial cells and different primary end-points to measure the levels of apoptosis (53). The nature of the mutation responsible for renal cystic disease may be relevant as well.

Apoptosis and apoptotic pathways in animal models resembling ARPKD

The cpk mouse develops renal cysts via the disruption of a cilia-associated protein, cystin (59). In kidneys from cpk mice, widespread apoptosis is detected in the interstitium, while few apoptotic cells are found in cystic epithelia or noncystic tubules (59). Since the activity of caspase-3 was increased in cpk mice (59) and knockout of caspase-3 in these mice delayed cyst growth and prolonged survival compared to the control mice (mean survival of 117 days versus 32 days, $P < 0.01$), it seemed that caspase-3 mediated apoptotic signaling might somehow promote cyst growth in cpk mice. However, the findings that apoptotic index, the expression level of Bcl2, BAX and caspase-7 showed no difference in cpk mice

with or without knockout of caspase-3 (51), contradicting this conclusion. In addition, another study found that inhibition of the Pax2 gene, which is essential for the differentiation and proliferation of the renal epithelium, inhibited renal cyst growth in Pax2 heterozygous cpk mice due to increased apoptosis but not reduced proliferation of cystic epithelium (60, 61). These results suggested that apoptosis might have more complicated roles in cpk mice than its proposed role in promoting cyst growth.

Juvenile cystic kidneys (jck) mice, which have a missense mutation in the Nek8 gene encoding serine/threonine kinase, are fertile and generally survive to four or more months of age. In kidneys from jck mice, apoptotic nuclei were very common in cystic epithelia, but few were seen in normal tubular epithelial cells (62). Therefore, jck cyst enlargement was accompanied by a high rate of cystic renal epithelial cell proliferation and increased apoptosis. Apoptosis in jck mice may be mediated by the pro-apoptotic protein Apaf1 and caspase-2 as well as Bcl-2 and Bcl-xL (63).

The PCK rat, an orthologous model for human ARPKD, has many features that resemble human ADPKD, such as focal development of cysts, although the pattern of inheritance is autosomal recessive. In the PCK rat, apoptotic cells are commonly found in the normal tubules and dilated tubules, but are less observed in cysts lined by flat epithelium (64). Apoptosis in the PCK rat may be mediated by p38 mitogen activated protein kinase (MAPK) signaling and caspase-7 but not caspase-3 (65).

Another example of a recessive model in which apoptosis was shown to contribute to cyst formation is the knockout mice for AP-2 β (a transcription factor), which develop numerous, small cysts in the distal tubule and collecting duct. In the AP-2 β knockout mice, embryonic development was completed but the mice died at postnatal day 1 or day 2 due to the PKD. In this case, there was increased apoptosis in collecting duct and distal tubular epithelia (66). The expression of antiapoptotic proteins Bcl-X(L), Bcl-w and Bcl-2 in AP-2 β knockout mice was downregulated at the end of embryonic development, which suggest the apoptosis induced by loss of AP-2 β may be dependent on the antiapoptotic Bcl-2 protein (66).

Bcl-2 as an antiapoptotic protein inhibits the activity of proapoptotic proteins BAX and BAK. Bcl-2-deficient mice also develop polycystic kidneys accompanied by abnormal apoptosis of the kidney tubular epithelium and interstitium (67-69). Bcl-2 knockout mice reassemble oligomeganephronic hypoplasia (70), which presents hypoplastic kidneys in the embryonic stage due to a reduced number of nephrons mediated by excessive apoptosis. In Bcl-2 knockout mice, apoptosis appears to be mediated primarily by the unopposed proapoptotic activity of the BH3-only protein Bim as deletion of a single allele

Apoptosis in ADPKD

of Bim is sufficient to abolish PKD in this model (71). This suggests that apoptosis is the essential driver of cystogenesis in Bcl-2 knock out mice (71).

In summary, abnormal apoptosis was detected in experimental ARPKD animal models, including cpk mice (59), jck mice (62), PCK rats (64), AP-2 β ^{-/-} mice (66), and Bcl-2 knockout mice (67). The mechanisms of apoptosis in these animal models are regulated through intrinsic Bcl-2 family member-mediated pathways. The involvement of extrinsic pathways in these animal models has not been reported thus far.

Apoptosis and apoptotic pathways in animal models resembling ADPKD

Increased apoptotic cells are also shown in Han:SPRD rats and c-Myc transgenic SBM mice that resemble ADPKD. The heterozygotes of Han:SPRD male rats (Cy/+) develop renal cysts and renal failure over several months, while homozygous (Cy/Cy) animals die in a few weeks post-birth due to the rapidly progressive renal enlargement (72). The TUNEL-positive apoptotic cells were increased in two week old heterozygous (Cy/+) and homozygous (Cy/Cy) rat kidneys compared to normal littermate controls and more than half of the apoptotic cells were from cystic tubules in kidneys of Han:SPRD rats (73). The activity of caspase-3, caspase-7 and caspase-8 is increased in the kidneys of homozygous Han:SPRD rats (Cy/Cy) compared with wild type rats (+/+), while it shows no difference in the kidneys from heterozygous Han-SPRD rats (Cy/+) and wild type rats (+/+) (73, 74). However, administration of the pan-caspase inhibitor IDN-8050 could slow disease progression by reducing tubular proliferation and apoptosis in the heterozygous Han-SPRD rats (Cy/+) (75).

c-Myc, an oncogene involved in cell proliferation, apoptosis, differentiation and neoplasia, has been found to be upregulated in human ADPKD and PKD animal models, including the Han:SPRD rat (Cy/Cy) (72, 76-79). The c-Myc transgenic SBM mouse developed polycystic kidney disease as displayed by elevation of proliferation index (10-fold) and apoptotic index (10 to 100-fold) in kidneys compared with nontransgenic controls (80-82). In c-Myc transgenic mice, apoptosis was induced by c-Myc-mediated activation of the pro-apoptotic protein Bax, leading to the release of cytochrome c from the mitochondria to the cytosol (83). However, c-Myc induced-apoptosis in polycystic kidney disease is Bcl-2 independent as overexpression of both Bcl-2 and c-Myc in vivo produce a similar PKD phenotype with a high apoptotic index compared to overexpression of c-Myc only (81). Although the expression of FasL was elevated in the c-Myc transgenic mouse kidneys, mutation of FasL in these mice was incapable of affecting apoptosis, which suggests that c-Myc-induced apoptosis in PKD is independent of FasL/Fas signaling (84). Because c-Myc has been reported to be upregulated in cystic epithelia from Han:SPRD rats (72), the

involvement of c-Myc signaling in regulating apoptosis in these rats needs to be investigated further.

Apoptosis in Pkd1 or Pkd2 mutant animal models

It is controversial as to whether abnormal apoptosis occurs in Pkd1 or Pkd2 mutant mouse models. In Pkd1^{-/-}/LZ⁺ chimeric mice generated by aggregation of Pkd1^{-/-} ES cells and Pkd1^{+/+} morulae from ROSA26 mice, the cyst epithelia of the kidney were composed of both Pkd1^{-/-} and Pkd1^{+/+} (Pkd1^{-/-}/LZ⁺) renal tubular epithelial cells in the early stages of cystogenesis. During cyst development, Pkd1^{-/-} cyst epithelial cells became dominant due to increased proliferation, which gradually replaced Pkd1^{+/+} (Pkd1^{-/-}/LZ⁺) cyst epithelial cells due to JNK-mediated apoptosis. It is important to note that, in this animal model, apoptosis was observed mostly in Pkd1^{+/+} (Pkd1^{-/-}/LZ⁺) cyst epithelial cells but less in Pkd1^{-/-} cyst epithelial cells (85) and was decreased when cyst enlarged, which suggested that if apoptosis contributed to cystogenesis it should be effect at early stage of cyst development.

However, in Pkd1^{flox/flox}:Tamoxifen-Cre mice, induction the deletion of Pkd1 before postnatal day 13 results in severely cystic kidneys within 3 weeks, while deletion of Pkd1 after postnatal day 13 results in cysts only after 5 months. However, only a small number of apoptotic cells in the medulla and cortex were observed in cystic kidneys from Pkd1^{flox/flox}:Tamoxifen-Cre mice but showed no difference in the kidneys from developing stage to adult stage, which suggests that apoptosis may not be the primary factor of cystogenesis (86).

Furthermore, recent studies indicated that, although deletion of *Pkd1* results in an incremental increase in cell proliferation in the *Pkd1* conditional knockout Pkd1^{flox/-}:Ksp-Cre mice, *Pkd1*^{flox/-}:Pkd1-Cre mice and Pkd1 hypomorphic Pkd1^{nl/nl} mice, increased apoptosis was not a feature in kidneys from these mice as the TUNEL-positive nuclei are negligible and had no difference between cystic and non-cystic kidneys (53, 54, 57, 87). This provided further evidence that apoptosis is not the primary factor of cystogenesis at least in Pkd1 knockout animals.

The role of apoptosis in cyst formation in Pkd2 mutant mice remains unclear because of conflicting reports. Increased apoptosis was observed in Pkd2 transgenic mice and in Pkd2 knockout renal cells isolated from Pkd2 conditional knockout mice (88, 89). Calcium influx mediated by transient receptor potential (TRP) channels in the plasma membrane triggers the cell death (90). PC2, also known as TRPP2, is enriched in the endoplasmic reticulum (ER) membrane. ER-resident PC2 reduces the Ca²⁺ release from ER and then decreases

Apoptosis in ADPKD

cytosolic and mitochondrial Ca^{2+} signals, which results in the protection from apoptotic stimuli (91). However, an opposite apoptotic phenomenon was reported in $\text{Pkd2}^{\text{WS25}}$ mice, in that Stroope et al. found that the apoptotic indices were increased in kidneys of $\text{Pkd2}^{\text{WS25}}$ mice compared with those in wild type mice (92), while Wei et al. found that apoptosis was not increased in these mice compared to the controls (54). In summary, increased apoptotic cells are shown in Han:SPRD rats and c-Myc transgenic SBM mice that resemble ADPKD. However, recent studies suggest that apoptosis may not be the predominant factor of cyst expansion in ADPKD.

Apoptotic pathways in ADPKD

As we described above, the extrinsic pathway-mediated apoptosis involves the death ligand, death receptors, initiator caspase-8 and the common downstream executioner caspase-3 and caspase-7. Caspase-3 and caspase-8 are activated in the small cysts and normal-appearing tubules but are not seen in the larger cysts in human ADPKD (93), which implies the involvement of these caspases in regulating apoptosis in the small cysts and normal-appearing tubules in human ADPKD. The death ligand TNF- α and death receptor TNFR1 are upregulated in the Pkd1 mutant cystic renal epithelial cells, however, the role of TNF- α /TNFR1 signaling is to inhibit apoptosis in these cells via association with the upregulated cIAP1, RIP1 and TRADD to form the pro-survival complex I (53, 94), leading to activation of NF- κB and the upregulation of c-FLIP to inhibit caspase-8. Thus, the involvement of the extrinsic pathway in mediating apoptosis associated with human ADPKD needs be investigated further.

The intrinsic pathway-mediated apoptosis involves caspase-9, cytochrome c and the Bcl-2 family proteins. Abnormal apoptosis in the kidney from homozygous Han-SPRD rats (Cy/Cy) may be mediated by decreased expression of anti-apoptotic Bcl-XL and Bcl-2 compared with wild type rats, leading to the release of procaspase-9 and cytochrome c into the cytosol (73). Increased apoptosis in c-Myc transgenic SBM mice (81) may also be mediated via Bcl-2 family proteins. However, studies by Hughes et al. demonstrated that loss of Pkd1 and loss of Bcl-2 elicit cyst formation through distinct mechanisms (95). They found that ablation of one or both alleles of the pro-apoptotic gene Bim prevented cyst formation in mice deficient for Bcl-2 while loss of Bim had no effect on cyst development in Pkd1 homozygous mutant mice. Nor did loss of Bcl-2 alleles significantly influence the Pkd1 mutant phenotype (95). These studies suggest that Bcl-2 mediated apoptosis is not involved in cystogenesis in mice with Pkd1 deficiency.

In addition to Bcl-2, the changes of the other members of the IAP family protein, cFLIP, cIAPs and survivin, have been observed in ADPKD. cFLIP inhibits the activation of

caspase-8 due to its structure similarity. cFLIP can only be detected in large cysts, whereas caspase-8 can be detected in small cysts and normal appearing tubules in end stage human ADPKD kidneys, which may explain why apoptosis can be observed in small cysts and normal-appearing tubules but not in large cysts (5). The expression of cFLIP is upregulated in Pkd1 mutant cystic renal epithelial cells and mouse kidney tissue, and can be induced by TNF- α , which constantly exists in cyst fluid (53, 94). The upregulated cFLIP in turn inhibits the TNF- α /TNFR1 mediated extrinsic apoptotic pathway in Pkd1 knockout mouse kidneys (53). In addition to cFLIP, cIAP1 is also upregulated in Pkd1 mutant cystic renal epithelial cells and kidney tissues, and is induced by TNF- α (53). The upregulated cIAP1 associates with TNF- α /TNFR1, TRADD, TRAF2 to form the pro-survival complex I to activate NF- κ B signaling, resulting in the survival of cystic renal epithelial cell (53). The TNF- α /TNFR1-mediated extrinsic apoptotic pathway can only be triggered until cIAP1 is degraded by the pro-apoptotic protein Smac or Smac-mimetics (53). Survivin, as another IAP, is undetectable in normal adult tissue but is expressed in cancer, such as renal cancers (96). Survivin inhibits the intrinsic apoptotic pathway via inhibition of Smac (97), and interaction with caspase-9 (96). Survivin is increased in cystic kidneys from Han-SPRD rats with increased caspase-9 and apoptosis (98). Thus, it is unlikely that survivin could inhibit the intrinsic apoptotic pathway in Han-SPRD rats. In contrast, AbouAlaiwi et al. reported that the expression of survivin was decreased in human ADPKD kidneys and kidneys from PKD mice (99, 100). In mouse and zebrafish models, deletion of survivin resulted in the cystic phenotype, which may be due to the abnormal oriented cell division since survivin plays an important role in regulating ploidy, in addition to regulating apoptosis (100).

The role of apoptosis in ADPKD

Apoptosis may act as a double-edged sword in human ADPKD. Apoptotic loss of renal tissue may be responsible for the progressive deterioration of renal function (49). Apoptosis detected in the normal-appearing, non-cystic tubules may result in the progressive loss of normal nephrons in PKD, which may be due to the direct compression from adjacent expanding cysts, apoptotic stimuli secreted by cystic epithelia and inflammatory cells. On the other hand, increased apoptosis in ADPKD may counteract hyperproliferation, which may prevent kidneys from progression of PKD into the renal cell carcinoma despite the high rate of epithelial cell proliferation. Recent retrospective study showed that cancer incidence was lower in PKD renal transplant recipients than in non-PKD renal transplant recipients (101). The exact mechanisms of the low cancer risk in PKD recipients are unclear, but may be associated with the increased apoptosis.

Cell model systems for the investigation of apoptosis and cyst formation

Early studies to delineate the role of apoptosis on cystogenesis involved Madin Darby canine kidney (MDCK) cell cultures. Apoptosis was proposed to be important for lumenization and other morphogenetic processes in kidney development and this prediction was supported by the following studies. The cyst cavitation of MDCK cells in a collagen-type I matrix resulted from apoptosis, and mimicked the proapoptotic state during renal development (102). It has been reported that MDCK cells overexpressing PC1 are resistant to apoptosis induced by serum starvation and are decreased in cell growth, which results in the formation of the branching tubule but not the simple cysts as in control cells (52). Studies also suggest that PC1 was able to inhibit apoptosis, in that: 1) MDCK cells overexpressing PC1 were resistant to G α 12 stimulated apoptosis, which is through the JNK activation and Bcl-2 degradation (103, 104); 2) knockdown of nephrocystin-1 increased apoptosis in PC1-overexpressed MDCK cells stimulated with TNF- α , which suggested that the interaction of PC1 with nephrocystin-1 was required for overexpression of PC1-mediated resistance to apoptosis in these cells (105); and 3) in 3D cell culture, apoptosis measured by staining for cleaved caspase-3 was detected in cyst lining cells in the large and spherical cysts formed by Pkd1^{-/-} cells, apoptosis was not detected in the extended, tubule-like structures formed by control Pkd1^{flox/-} cells, and reintroducing PC1 c-terminal tail (CTT) can suppress apoptosis in this system (106). In addition, Lin et al. reported that overexpression of the anti-apoptotic protein Bcl-2, which is essential for survival of renal stem cells during nephrogenesis, inhibited the cystogenesis in the MDCK system by inhibiting apoptosis (107). These studies, together with the findings that abnormally increased apoptosis was observed in animal models resembling ADPKD and ARPKD, suggest that apoptosis may be causally linked to the development of renal cystic disease. However, recent studies that apoptosis is negligible in kidneys from Pkd1 mutant mice challenges the role of apoptosis in Pkd1 knockout-mediated cyst development.

The relationship of apoptosis and cystic renal epithelial cell proliferation in PKD

PKD has been labeled “neoplasia in disguise” since the aberrant cell cycle progression and hyperproliferation are similar to cancer development (108,109). Hence, if hyperproliferation is a predominant factor that contributes to cystogenesis, it is important to understand the relationship of proliferation and apoptosis in the pathogenesis of ADPKD. Positive and negative correlations between apoptosis and proliferation have been reported in other diseases. For example, increased apoptosis and proliferation have been identified in dextran sulfate sodium-induced colitis (110) and neoplastic transformation of the colon (111) as well as in the progression of prostatic intraepithelial neoplasia (PIN) (112). In comparison, decreased apoptosis has been reported during progression from

adenoma to carcinoma in the colon (111) as well as from intraepithelial neoplasia to carcinoma in the human prostate (113), suggesting apoptosis may oppose proliferation and may be an important determinant of net tissue growth. The upregulation of c-Myc signaling in human ADPKD and PKD animal models (72, 76-79) may act as a model to explain the positive relationship between apoptosis and proliferation in PKD. In addition to its growth promoting activity, c-Myc is also an apoptosis inducer under stress conditions (114). c-Myc promotes apoptosis by triggering the release of cytochrome c from mitochondria and by activating caspase-9, and c-Myc-induced apoptosis can be inhibited by the antiapoptotic protein Bcl-2 (114, 115). In cancer cells, overexpressed Bcl-2 potentiates the oncogenic action of c-Myc by allowing c-Myc-induced proliferation to proceed without apoptosis (116), which suggests that apoptosis may act as part of a cellular fail-safe mechanism to forestall continued proliferation (114, 115). Thus, apoptosis in cystic epithelium may also act as a protective mechanism to limit the consequences of aberrant proliferation. In contrast, recent evidence suggests that caspases and other proapoptotic proteins can induce proliferation of neighboring surviving cells to replace dying cells through apoptotic cells secreting mitogens, such as Wg and Dpp mediated by JNK and dp53, in *Drosophila* (117). This process is called "apoptosis-induced proliferation", and it may be critical for tissue regeneration and tumor repopulation during cancer irradiation (117). Whether tubular cell apoptosis contributes to increased cell proliferation of neighboring cysts and tubules is not determined.

Although proliferation of cyst lining epithelia was increased, no obvious apoptosis was detected in several Pkd1 conditional knockout mice and Pkd1 hypomorphic Pkd1^{nl/nl} mice. Growing evidence supports a mechanism that slowing cyst growth is through cell cycle arrest and restoration of pro-apoptotic activities to induce cell death, which is similar to some effective anti-cancer therapies (114). For example, inhibition of SIRT1 with nicotinamide or EX-527 decreased cystic renal epithelial cell proliferation by inactivation of p-Rb, and induced its apoptosis by activating p53 in a Pkd1 conditional knockout mouse model (58). In addition, inhibition of macrophage migration inhibitory factor (MIF) with ISO-1 decreased cystic renal epithelial cell proliferation but increased apoptosis (57). However, the direct role that inducing apoptosis within proliferating cystic renal epithelium has in delaying cyst growth in ADPKD is not provided by these studies, since inhibiting cell proliferation may be the predominant effect. A recent report demonstrated, for the first time, that induction of cystic epithelial cell death with Smac-mimetics delayed renal cyst growth with no effect on cell proliferation (53). Smac-mimetics are cell-permeable compounds designed to mimic the N-terminal 4 amino acid of Smac, a mitochondrial protein that binds to and antagonizes IAPs, including cIAP1, cIAP2 and xIAP (97, 118). TNF- α together with a Smac-mimetic induced cancer cell death (97, 118). Smac-mimetics induced the degradation of cIAP1, leading to its disassociation from

complex I, which then promoted the formation of complex II to induce apoptosis of cystic renal epithelial cells (53). Treatment of $Pkd1^{flox/flox};Ksp-Cre$ mice and $Pkd1^{nl/nl}$ mice with Smac-mimetics strikingly delayed cyst growth and preserved renal function, which resulted from the increased apoptosis of cyst lining epithelia (53) (Figure 2). Thus, this study suggests that apoptosis does not contribute to cystogenesis in PKD1 animal models; rather, it reduces the expansion of cysts and may be a new therapeutic target for the treatment of ADPKD.

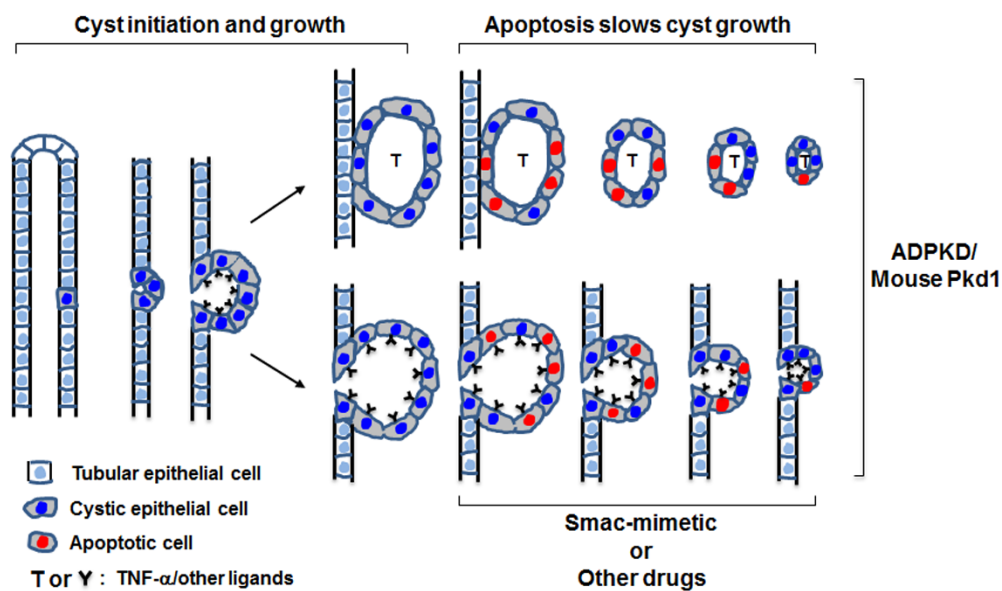


Figure 2. Model depicting the effect of Smac-mimetics or other potential drugs on delaying cyst enlargement in human ADPKD and ADPKD animals. Cysts arise primarily in collecting ducts when cell polycystin-1 levels fall to a critical threshold. A new phenotype arises that proliferates in response to cyclic AMP and other growth factors, and generates cytokines and chemokines, including TNF- α which has been found to accumulate in cyst fluid in an isolated sac formed after the cyst separates from the tubule in ADPKD patients (top panel). While the cyst remains attached to the tubule, some of the TNF- α synthesized in the mutant cells may escape into the urine but some of the TNF- α may retain at cyst developing sites through binding to its receptor, TNFR1 (bottom panel). TNF- α is trapped within the cysts or is trapped with TNFR1 to partner with Smac-mimetics to promote apoptosis of mural cells. Removal of apoptotic mural cells would reduce cyst surface area and decrease the rate of cyst growth. Extensive apoptosis could cause cysts to shrink. Other identified or unidentified drugs to target apoptotic pathways other than TNF- α signaling in ADPKD may shrink cyst or delay cyst growth in a similar manner.

Therapeutic strategies of regulating apoptosis in PKD

The therapeutic interventions involved in regulating apoptosis in PKD animal models are summarized in Table 1. Roscovitine is a cyclin-dependent kinase (CDK) inhibitor, and has shown promise in cancer treatment. Intermittent administration of roscovitine has a long-lasting effect to delay cyst growth in two non-orthologous mouse model of ARPKD, jck mice and cpk mice (63). The apoptotic cells were decreased in kidneys of roscovitine-treated jck mice, which is associated with the decreased expression of proapoptotic protein Apaf1 and caspase-2, and the increase expression of Bcl-2 and Bcl-xL (63). The mechanism of inhibiting the apoptosis by roscovitine may also involve the inhibition of Cdk5, which is responsible for anti-apoptotic effects of roscovitine in neurodegenerative diseases (63, 119). The GlcCer synthase inhibitor, Genz-123346, also slowed cyst growth by decreasing the proliferation and apoptosis in jck mice (120). The angiotensin converting enzyme inhibitor, Lisinopril (121), and water intake (65) have been reported to delay cyst growth associated with the decreased proliferation and apoptosis in the PCK rat. The studies in Han:SPRD rats also suggest that inhibiting proliferation and apoptosis with the caspase inhibitor IDN-8050 (75), soy protein feed (122), catechol-O-methyltransferase (COMT) inhibitor Tolcapone (123) and 2-hydroxyestradiol (124) can slow the cyst expansion. However, it is worth noting that decreased cell apoptosis is consistently accompanied with the decreased epithelial cell proliferation in all these studies. If hyperproliferation is the predominant factor for cyst expansion as described above, the decreased proliferation induced by these therapeutic interventions should be the primary effect on reduction of renal cysts.

The mammalian target of rapamycin (mTOR) pathway is an important pathway to regulate cell growth by sensing and integrating diverse nutritional and environmental signals, including growth factors (such as IGF-1 and IGF-2), energy levels, amino acids, and cellular stress (125, 126). PC-1 associates with tuberlin and mTOR to form a complex to downregulate mTOR activity in renal epithelial cells under normal condition (55). N-terminal cytoplasmic PC1 (NTM-PC1) acts a constitutively-active inhibitor of mTOR, since it can inhibit the mTOR activity which results in G1 cell cycle arrest and apoptosis (55). The mTOR pathway is aberrantly activated in cystic epithelial cells in human ADPKD and mouse PKD models (55). Rapamycin treatment reduces renal cysts in the orpk-rescue mutant mouse model, which is a late-onset form of PKD due to defects in cilia protein polaris encoded by Tg737 gene, and Pkd1 conditional knockout Pkd1cond/cond:Nestin-Cre mice, and reduces the size of affected kidneys in ADPKD patients after renal transplantation (55, 56). The selective induction of apoptosis and luminal shedding of cyst-lining epithelial cells by rapamycin may be one of the potential mechanisms to slow cyst growth (55). Overexpression of neutrophil gelatinase-associated lipocalin (NGAL) mediated by adenovirus suppressed renal cyst growth in Pkd1^{flox/-}:Ksp-Cre mice, partially due to the

Table 1. Therapeutic intervention involving in the regulation of apoptosis

Therapeutic intervention	Animal models	Mutated gene	Protein	Human disease	Proliferation	Apoptosis	Reference
Roscovitine, CDK inhibitor	jck mice	Nek8	Nek8	Nephronophthosis	decrease	decrease	(63)
Genz-123346,	cpk mice	Cys1	Cystin	NA	decrease	decrease	(120)
GlcCer synthase inhibitor	jck mice	Nek8	Nek8	Nephronophthosis	decrease	decrease	(121)
Water intake	pk rat	Pkhd1	Fibrocytin	ADPKD	decrease	decrease	(65)
Lisinopril, ACE inhibitor	pk rat	Pkhd1	Fibrocytin	ADPKD	decrease	decrease	(75)
IDN-8050, Caspase inhibitor	Han:SPRD rat	Anks6	Ankyrin repeat and SAM domain-containing protein 6	Nephronophthosis	decrease	decrease	(122)
Soy protein	Han:SPRD rat	Anks6	Ankyrin repeat and SAM domain-containing protein	Nephronophthosis	decrease	decrease	(123)
Tolcapone, COMT inhibitor	Han:SPRD rat	Anks6	Ankyrin repeat and SAM domain-containing protein	Nephronophthosis	decrease	decrease	(124)
2-Hydroxyestradiol, 17- β Estradiol metabolites	Han:SPRD rat	Anks6	Ankyrin repeat and SAM domain-containing protein	Nephronophthosis	decrease	decrease	(131)
PP242, mTOR kinase inhibitor	Han:SPRD rat	Anks6	Ankyrin repeat and SAM domain-containing protein	Nephronophthosis	decrease	decrease	(55)
Rapamycin, mTOR inhibitor	Orpk mouse	Tg737	Polaris	NA	NA	increase	(132)
mTOR anti-sense oligonucleotide (ASO)	Pkd2WS25/-	Pkd2	Polycystin 2	ADPKD	decrease	decrease	(56)
Rapamycin, mTOR inhibitor	Pkd1 ^{lox/lox} ; Nestin-Cre	Pkd1	Polycystin 1	ADPKD	decrease	increase	(54)
Neutrophil gelatinase-associated lipocalin	Pkd1 ^{lox/lox} ; Ksp-Cre	Pkd1	Polycystin 1	ADPKD	decrease	increase	(58)
Nicotinamide and EX-527, Sirtuin 1 inhibitor	Pkd1 ^{lox/lox} ; Ksp-Cre	Pkd1	Polycystin 1	ADPKD	decrease	increase	(57)
ISO-1, MIF inhibitor	Pkd1 ^{u/nl}	Pkd1	Polycystin 1	ADPKD	decrease	increase	(53)
Smae-mimetic	Pkd1 ^{lox/lox} ; Ksp-Cre	Pkd1	Polycystin 1	ADPKD	decrease	increase	(53)
	Pkd1 ^{u/nl}	Pkd1	Polycystin 1	ADPKD	No change	increase	

induction of apoptosis of cystic epithelial cells by sequestration of intracellular iron and subsequent activation of Bim1 (54, 127). In addition, as we described above, both SIRT1 inhibitors and the MIF inhibitor delayed cyst growth in Pkd1 knockout mice partially through induction of cystic epithelial cell death through the activation of p53 (57, 58), whereas Smac-mimetics slowed cyst growth mostly through disruption of the TNF- α mediated pro-survival NF- κ B signaling and then induction of cell death in cystic epithelium but not the non-cystic epithelial cells (53). These studies suggest that induction of cyst lining epithelial cell apoptosis, with or without a decrease in proliferation, can help to slow cyst growth in Pkd1 mutant animal models.

Conclusion and Perspectives

Understanding the potential detrimental and beneficial roles and mechanisms of apoptosis in PKD is necessary for developing therapeutic strategies in the future. Although aberrant apoptosis has been suggested as one of the pathological features in human ADPKD, ARPKD and PKD animal models, more recent evidence indicates that apoptosis is not increased in Pkd1 mutant mouse models, and induction of cyst lining epithelial cell apoptosis indeed slows cyst growth in these animals. Thus, it is necessary to thoroughly evaluate the role and mechanism of apoptosis during cyst development, which should include the origin of apoptotic cells (such as the cystic tubule cells, dilated tubule, normal tubule and interstitial cells), apoptosis in different stages of disease (such as the early stage of cyst initiation, intermediate stage of cyst expansion, and late stage of renal failure) and in different animal models (such as ARPKD and ADPKD models), and the relation of proliferation and apoptosis as well as its associated signaling pathways in PKD. It is possible that 1) apoptosis may be a secondary effect of aberrant cystic epithelial cell proliferation and is not directly related to the induction of cell proliferation; and 2) if we induce apoptosis of cystic cells we can overcome the increased cell proliferation and remove the mutant cells from contributing to cyst growth in the future. The pathogenesis of PKD is relevant to the hallmarks of cancer, such as the aberrant activation of signaling pathways of mTOR, Rb-E2F1, JAK/STAT (58, 128, 129). Several anti-cancer drugs have shown promise in ADPKD, such as rapamycin (56), Hsp90 inhibitor (130), and Smac-mimetics (53). Thus, novel therapeutic interventions may be identified for PKD treatment by repurposing the anti-cancer drugs (Figure 2) either to induce cell apoptosis alone or together with inhibiting cell proliferation.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 10

c-Myc Signalling in the Genetic Mechanism of Polycystic Kidney Disease

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Abstract

The Myc family of transcription factors regulates major biological processes such as proliferation, stem/progenitor cell pluripotency, metabolism, apoptosis, cell growth and differentiation. The most-studied member c-Myc is essential in embryonic development and cellular homeostasis. Dysregulation of c-Myc protein function is not only associated with malignant transformation and human tumors but is also implicated in autosomal dominant polycystic kidney disease (ADPKD), a human genetic disorder, considered a neoplasia in disguise. Studies from human ADPKD kidneys, caused by mutation in the

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PKD1 or PKD2 genes, revealed high expression of c-Myc with strong signal detected over cystic tubular epithelium. Consistent with human ADPKD pathogenesis, mouse models produced by dysregulation of Pkd1 and Pkd2 gene dosage show stimulation of renal c-Myc expression. Induced renal c-Myc expression is also observed in several non-orthologous animal models of PKD. Significantly, c-Myc overexpression specifically targeted to renal epithelial cells in transgenic mice closely reproduces human ADPKD. The specific causal effect of c-Myc in PKD was demonstrated by targeting different oncogenes which could not mimic the PKD phenotype. In fact, c-Myc was shown to be a major mediator of renal cystogenesis through various mechanisms and signalling pathways. Most importantly, inhibition of c-Myc *in vivo*, directly by repressing translation or indirectly with a small-molecule inhibitor, significantly delayed cystogenesis in the mouse. In summary, c-Myc is a central node in the pathogenesis of Pkd1/Pkd2 mouse models and of human ADPKD development and progression.

Key words: Mouse models; Myc; Polycystic kidney disease; Renal development; Signalling

Introduction

Autosomal dominant polycystic kidney disease (ADPKD), a ciliopathy, is characterized by formation of renal tubular cysts that involves increased epithelial cell proliferation and apoptosis, alterations in cell polarity and in tubular basement membrane and abnormalities in trans-epithelial fluid transport. Early on, ADPKD was claimed to be a neoplasia in disguise (1) and has been regarded as a multifocal neoplastic disorder. Molecular genetic analysis of ADPKD implicates one of two loci, PKD1 or PKD2. PKD1 encodes polycystin-1 (PC1), predicted to be a large cell surface receptor, and PKD2 encodes polycystin-2 (PC2), a non-selective calcium channel. Progress in understanding the molecular basis and pathogenesis of ADPKD was greatly enhanced by analyses carried out in murine experimental systems and on the PC1 and PC2 proteins. Studies on the ADPKD proteins have revealed that PC1 and PC2 interact together and with multiple proteins including other cystoproteins. Although the precise functions of the polycystin proteins are an issue not yet resolved, many signalling pathways and transcription factors are activated during ADPKD development and progression of cyst growth. A common key molecular pathogenic effector in the ADPKD network is c-Myc. In fact, there is overwhelming evidence for upregulation of c-Myc in renal cystic diseases. This chapter will address the role of c-Myc and its functional relationships in normal kidneys and ADPKD pathogenesis (2-4).

Insights into Myc biology

Myc is a pleiotropic transcription factor that regulates a multitude of cellular functions. This section gives an overview of its regulation and function at molecular and cellular level.

Regulation of Myc gene expression

c-Myc is among the best-studied proteins in biology. It is a member of a gene family that also includes N- and L-Myc, all of which are basic region/helix-loop-helix/leucine zipper transcription factors. These factors carry out similar functions that are dependent also on their spatial and temporal expression patterns (5). Myc is an immediate early growth response gene that is rapidly induced upon signalling from Wnt, Hedgehog, Notch and many receptor tyrosine kinases like extracellular signal-regulated protein kinase (ERK) (6-9). Expression of c-Myc is under tight regulation not only at the level of transcription and the mRNA itself but also post-translationally with modifications that provide stringent controls. Several transcription factors regulate c-Myc expression including the transcriptional regulator Brd4, which contains a bromodomain and extraterminal domain (BET) that binds to the c-Myc regulatory region (10, 11). Myc transcription is also controlled at both initiation and elongation by RNA polymerase II (12-14). Furthermore, the c-Myc transcripts are modulated by microRNAs (miR): miR-145, miR-34, miR-24 and Let-7 (15-20). The c-Myc protein can be degraded in the nucleus by the ubiquitin-proteasome system via several E3 ligases (21, 22) and it has a very short half-life of approximately 20 minutes (23).

More recently, c- and N-Myc were found to be post-translationally controlled by proteolytic cleavage (24). The Myc protein is cleaved in the cytosol by calcium-activated calpains that produce a protein termed Myc-nick, which lacks the C-terminal region essential for nuclear translocation (NLS) and DNA binding (bHLH, LZ) (24). Myc-nick binds to the microtubular cytoskeleton, and with the histone acetylase (HAT) GCN5 (25) mediates acetylation of α -tubulin involved in trafficking, primary cilia assembly and mitotic spindle formation. Hence, cleavage of Myc reduces levels of transcriptionally-active, nuclear Myc and produces Myc-nick which regulates microtubule dynamics and function to promote cell differentiation. Switching of full-length c-Myc to the cleaved form could explain the paradoxical roles of both inhibition and stimulation of cell differentiation attributed to c-Myc. Therefore, it is presumed that the proportion of full-length c-Myc converted into Myc-nick should be tightly regulated to determine lineage commitment and differentiation.

Myc molecular functions

c-Myc forms heterodimers with Max and together they bind DNA at one of the most frequent motifs in the genome, the canonical E-boxes. Genome wide mapping of Myc binding sites to chromatin and gene expression profiling identified a considerable number of potential gene targets (26). The Myc-Max heterodimer recruits multiple coactivator complexes and is associated with chromatin modification and gene activation. It is well recognized, however, that Myc gene activation is notoriously modest. In fact, all Myc target genes are not necessarily transcriptionally-responsive. Many studies have shown that Myc cooperates with a diverse set of additional factors to influence expression of target genes. Importantly, Myc promotes transcriptional regulation (27) in a dose-dependent fashion, such that as levels of Myc increase, gene activation becomes more generalized. Myc has been found to operate via a novel transcriptional mechanism, the “amplifier model”, that consists of the transcriptional amplification of gene expression already in an activate state to coordinate a growth program (28, 29). As in the case of many transcriptional factors, Myc can also play a role in transcriptional repression when complexed with Miz-1 or through recruitment of histone deacetylases (HDACs). An additional mode of Myc regulation is through a network of microRNAs. c-Myc activates expression of a locus of 6 microRNAs, miR-17~92 via direct binding (30) and represses expression of several microRNAs, miR-23, miR-29, Let-7 (31-33).

Myc cellular functions

The Myc proteins are involved in major biological processes including proliferation, metabolism, cell cycle progression, apoptosis, cell growth and differentiation, fibrosis, and polarity (34-36) (Figure 1). All of these cellular functions are altered and ongoing in ADPKD. Consistent with the cooperative function of c-Myc with other transcription factors in stem cells, c-Myc was shown to promote and maintain pluripotency of stem and progenitor cells (37, 38). While high expression of c-Myc is essential in embryonic development, Myc expression levels normally wane in late developmental and cell differentiation stages.

When upregulated, the c-Myc protein is a potent oncogene involved in malignant transformation and in most types of human tumors (39). The Myc family members, in contrast to several oncogenes, do not need to undergo changes in coding sequence. In fact, Myc overexpression can drive a series of cellular changes, de novo mutations, and genomic instability that promote human malignancies. The c-Myc oncoprotein contributes to the genesis of different forms of human cancer ranging from lymphomas

to solid tumours (40, 41). N-myc is frequently overexpressed in solid cancers like neuroblastoma (42, 43) and L-Myc in small cell lung carcinomas (44). Myc contributes to tumorigenesis through various mechanisms. By compelling transcriptional regulation of many E-box genes and/or non-transcriptionally, Myc can play a role in the stages of initiation, maintenance, and progression of tumorigenesis, which led to the concept of “Myc addiction” (45). This Myc addiction can result, at least in part, from the need for energy metabolism since Myc regulates different genes of the glutaminolytic and glycolytic pathways (32, 46-48).

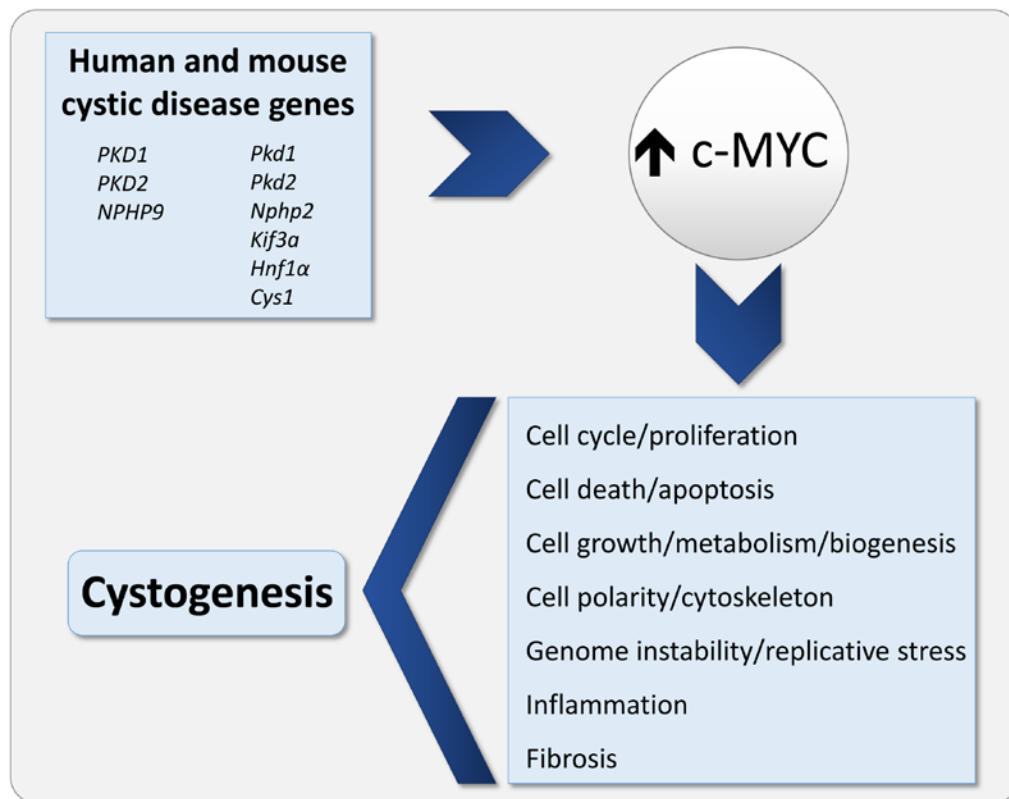


Figure 1. c-Myc at the node of PKD signalling. This schematic representation shows a cystogenic mechanism that could be operative in PKD. The c-Myc protein is depicted at the centre of the PKD pathogenic mechanism. Human ADPKD and nephronophthisis as well as cystic mouse models, orthologous and non-orthologous, induce upregulation of c-Myc expression. The well-studied c-Myc protein functions to elicit key cystogenesis features.

Role of c-myc in kidney development and homeostasis

Myc is central to the formation of the metanephros, the definitive kidney that initiates from embryonic day 10.5 (e10.5) in mice. Renal organogenesis involves mutual cellular inductive interactions between the metanephric mesenchyme and the ureteric bud that incite a subset of metanephric mesenchyme cells to condense and form the cap mesenchyme where stem cells are localized (49). The Myc family members are expressed during nephrogenesis but have distinct cellular patterns of expression in renal development. Normally, endogenous c-Myc is expressed early in uninduced renal mesenchyme, but also in the comma-shaped bodies, S-shaped bodies, and elongating tubules from e11.5-16.5. Upon renal maturation, c-Myc expression decreases to undetectable levels postnatally in differentiated renal tubular epithelium (50, 51). In comparison to c-Myc, N-Myc expression is even more transient with high levels upon renal mesenchyme induction, and is undetectable once the transition to epithelium is completed, whereas L-myc expression is confined to epithelium of the ureteric bud derivatives, in particular the collecting ducts (50).

Insights on the role of the Myc genes in the kidneys were provided from genetic studies using gene targeting. Mice with targeted disruption of the L-Myc gene were viable and had no renal histological anomalies (52), suggesting that L-Myc plays no essential role in the kidneys. Inactivation of N-Myc is embryonic lethal between e10.5-e12.5 with underdeveloped heart and neuronal defects (53). Embryos with a hypomorphic N-Myc allele resulted in hypoplastic kidneys at e13.5 with normal developing structures, due to reduced cell proliferation in mesenchymal cells and ureteric bud (54). Germline c-Myc inactivation leads to small and developmentally-retarded embryos that die at e9.5-e10.5 (55), and similarly epiblast-restricted c-Myc conditional inactivation resulted in fetal demise at e11.5 (56, 57) which precluded further analysis of c-Myc function in renal development as well as in adult renal homeostasis.

The role of c-Myc during renal development and homeostasis was determined by using a conditional c-Myc allele (58) at two distinct stages of development (2). Inactivation of c-Myc in early metanephric mesenchyme (e11.5) (59) showed that c-Myc plays an early and crucial role in the renal cap mesenchymal cells. Ablation of c-Myc resulted in depletion of the renal stem/progenitor cell population caused by a major decrease in proliferation that likely impaired their self-renewal potential (2). Interestingly, loss of c-Myc leads to a significant reduction of kidney size but with a normal branching pattern, cellular commitment, and architecture, revealing the preservation of normal renal developmental cues or developmental program. In fact, this renal c-Myc inactivation reproduced the typical pathologic condition of renal hypoplasia. In contrast, ablation of c-Myc at a later stage of renal epithelialisation (e17.5) (60) determined that c-Myc deficiency from e17.5

onward had no detectable impact on renal differentiation, maturation or homeostasis (2). This finding indicated that c-Myc is not essential from the late phase of renal development to adulthood, consistent with barely detectable c-Myc expression in mature kidneys. Hence, c-Myc is only essential in the cap mesenchymal cells within a critical developmental window. Therefore, the presence of c-Myc expression in mature kidneys likely results from stress signals (e.g. metabolic, DNA damage), environment stimuli (ischemia) or genetic conditions and could play a critical role in renal pathologies like PKD. Given that renal homeostasis can occur in a c-Myc independent manner, this implies that abrogation of renal c-Myc expression specifically can be a therapeutic strategy to prevent or stop the development of human adult pathologies such as PKD or renal cell carcinoma.

Upregulation of c-myc in human ADPKD

ADPKD pathogenesis in humans is presently thought to be a gene dosage dependent mechanism based on characterization of mouse models (61). In ADPKD patients, expression levels of the PKD1/PKD2 mutated allele can be strongly or mildly reduced. Paradoxically, PKD1/PKD2 and PC1/PC2 are found overexpressed in human ADPKD kidneys (3, 62-67). The severity of the ADPKD cystogenic mechanism(s) is likely to depend on modulation of the remaining non-mutant copy of PKD1/PKD2 potentially through de novo mutations, a stochastic event, altered functional role of polycystins and/or epigenetic inheritance that are actively under study.

Nonetheless, signalling pathways, networks and downstream effectors have been investigated in human ADPKD. Quantification of c-Myc and N-Myc expression in renal tissues from ADPKD patients revealed that all human ADPKD kidney biopsies showed highly elevated c-Myc levels (up to 15-fold) whereas N-Myc expression is unaffected (68). Analysis from ADPKD kidney biopsies using microarrays confirmed increased c-Myc expression (69, 70). Significantly, expression levels of c-Myc in fetal ADPKD kidneys were increased by approximately 40-fold, indicating that c-Myc is induced in both early and advanced stages of ADPKD. In addition, expression of c-Myc was detected by a strong signal intensity over renal ADPKD cystic epithelial cells by in situ hybridization (3). High expression of c-Myc indicated an active state of c-Myc during cystogenesis and correlated with a role in disease development and/or progression (Figure 1). Consistent with c-Myc function, ADPKD kidneys undergo increased epithelial proliferation (10 to 100-fold) and show an elevated apoptotic index (up to 100-fold) mainly localized in the cystic epithelium (3, 71). The apoptotic pathway involved is most likely independent of p53 and Bcl-2 since the expression levels of the pro-apoptotic p53 were low to undetectable whereas those of the anti-apoptotic Bcl-2 increased ~10 to 20-fold (3). Both proliferation and apoptosis in

epithelial cysts demonstrated a focal distribution with frequent cell clusters, implicating paracrine regulation via cell-cell interaction or disruption of basement membrane via cell-matrix interaction.

Several genetic diseases cause renal cystic disease in human. Among them nephronophthisis 9 or NPHP9/NEK8 was found to downregulate expression of the orthologous PKD1 and PKD2 genes and to increase c-Myc expression levels (72) (Figure 1). Other ciliopathies including other nephronophthises, Bardet-Biedl syndrome, Meckel-Gruber syndrome and oro-facial-digital type1 syndrome develop renal cysts but the molecular role and intracellular interactions remain to be investigated for a potential association with Myc dysregulation in these conditions.

Upregulation of c-Myc in renal cysts of human ADPKD results most likely from dysregulation of key developmental signalling pathways. Insights into possible human polycystin networks and downstream effector pathways were acquired from global and candidate approaches on human ADPKD kidneys and cell lines. These studies reported activation of developmental pathways including Wnt, Sonic Hedgehog, Notch, Hippo, bone morphogenic protein/transforming growth factor- β (BMP/TGF β), ERK and transforming growth factor- α (TGF α) signaling (69, 70, 73-75) that are known to regulate the c-Myc early growth response gene (6-9). Studies from human ADPKD tissues pointed to one or several signaling pathways that converge on c-Myc as a key “cystogenic” factor. Consistent with c-Myc being a central node in ADPKD, renal cellular energetics in ADPKD and even in the autosomal recessive form of PKD, ARPKD rely on activation of the glutaminolytic and glycolytic pathways, known to be c-Myc molecular metabolic targets in cancer (70, 76).

c-Myc, a key cystogenic factor in murine PKD

Presently, it is thought that c-Myc upregulation is a hallmark of PKD and cystogenesis in general. c-Myc is almost universally upregulated in cystic kidney diseases and virtually, independently of the underlying mutated genes.

c-Myc as an inducer of PKD in the SBM mouse model

While several mouse models with c-Myc overexpression promote carcinogenesis in collaboration with other factors as p53 and the Bcl2 gene family, c-Myc upregulation in the kidneys is cystogenic (4, 77) and reproduces human ADPKD as evidenced by the SBM mice (4). The unique SBM transgenic mouse model was generated by expression of the murine

c-Myc gene driven with the “SB” regulatory elements that target specifically renal epithelial cells. Mice derived from the 18 independent SBM transgenic lines consistently developed a PKD phenotype with 100% penetrance (4, 68, 78-81). The SBM mice exhibit all of the typical PKD renal morphologic and physiologic features. SBM kidneys are enlarged with numerous tubular and glomerular cysts that initiate in utero from e16.5 and progress with age. All nephron segments are affected in SBM kidneys as observed in human ADPKD (79). Developing cysts displayed extensive glomerular and tubular epithelial hyperplasia, in particular in fetal and young kidneys (postnatal day 0 to 20). Consistently, SBM kidneys have shown a markedly increased renal proliferation index of ~5 to 20-fold (78) and increased apoptosis of ~3 to 10-fold. The proliferation and apoptosis frequently occurred in cell clusters, suggesting cell-cell and/or cell-matrix interactions. SBM renal epithelia exhibit abnormal cell polarity by mislocalization of Na/K-ATPase, fodrin, ankyrin, and E-cadherin, and even loss of marker identity for a minority cystic tubules (79, 80) (Figure 1). These cellular characteristics could be compatible with a conflict in signals elicited from elevated full-length c-Myc and Myc-nick levels, resulting in microtubule hyperacetylation and altered cell differentiation (24). The SBM cystic kidneys showed persistence of immature renal epithelium either undifferentiated or dedifferentiated (80). As also found in ADPKD patients, SBM mice develop high blood pressure (~200mmHg) associated with vascular abnormalities (81). Interestingly, SBM renal parenchyma displays regions of fibrosis and focal infiltrates. SBM kidneys show evidence of renal epithelial chromosomal abnormalities, multipolar spindles, adenomas at young age, but no adenomas or carcinomas in adulthood (78). Consequent to the pathologic features, SBM died of renal insufficiency at ~3 to 5-months with severe renal damage and proteinuria.

The importance and specificity of c-Myc in PKD development and renal proliferation was demonstrated by several studies. First, spontaneous revertants in several SBM transgenic lines with mutations or partial deletions of the transgene in germinal cells were generated that did not develop a PKD phenotype (82). The absence of PKD demonstrated that the intact c-Myc transgene is necessary and sufficient to produce the SBM phenotype. In addition, the specificity of c-Myc in PKD was shown by substitution of c-Myc in the SBM transgene by the c-Fos early response gene and well-characterized proto-oncogene, linked to the “SB” regulatory elements producing the SBF transgene. None of the mice from the eight SBF transgenic lines developed renal abnormalities despite high levels of transgene renal expression (68). Furthermore, the c-Myc gene in the SBM transgene was also substituted by the well-studied growth factor TGF α coding sequence (68). The five SBT transgenic lines generated had no gross or microscopic renal abnormalities. These findings showed that the upregulation of c-Fos and TGF α in cells of human ADPKD cysts (69, 70, 83, 84) are not necessarily causative. Moreover these studies indicated that the PKD

phenotype in SBM mice depends on the specific functions inherent to c-Myc itself and not simply on a general mitogenic deregulation of the renal epithelial cells (4, 82).

In addition to proliferation, c-Myc was shown to promote cellular apoptosis, a prevalent process in PKD (3) (for review (85)) (Figure 1). c-Myc can function by altering a number of pro- and anti-apoptotic molecular mechanisms, in particular p53, Fas, and members of the Bcl2 gene family (86-88). Consistently, SBM kidneys showed increased programmed cell death affecting most severely the tubular cystic epithelium (68). The apoptotic mechanism induced by c-Myc in SBM mice appears independent of the well-known pathways. Successive matings of SBM mice with p53-null mice generated several p53^{-/-} SBM mice. All of these adult mice developed renal tubular cysts similar to SBM, died at the same age, had similar renal apoptotic index, and had no evidence of carcinomas. Transgenic mice carrying the major suppressor of apoptosis Bcl-2 linked to the "SB" regulatory elements expressing high Bcl-2 transgene levels mated to the SBM lines revealed that Bcl-2 expression did not modify the apoptotic rate, slow down PKD or even develop carcinomas (68, 89). Moreover, the SBM mice with c-Myc induced apoptosis were not rescued even partially with a deficient FasL pathway (89). Collectively, the SBM apoptotic mechanism is independent of p53, Bcl-2 and FasL/Fas, consistent with the human ADPKD studies. This novel and atypical *in vivo* c-Myc apoptotic pathway may play a critical role in PKD and potentially in the various human cystic diseases. As such, the SBM transgenic mice demonstrated a definitive causal connection between c-Myc and cyst formation in PKD.

c-Myc, a critical regulator in non-orthologous cystic mouse models

One of the most extensively studied spontaneous murine model of PKD is the autosomal recessive congenital polycystic kidney (cpk) mutant that closely resembles ARPKD. The cpk gene encodes Cystin-1 that is expressed in the cilia of collecting duct epithelia (90). Significantly, the cpk mice were shown to have elevated levels of c-Myc expression (91) and of the c-Fos and Ki-ras proto-oncogenes in kidneys (92). Interestingly, metabolomic analyses on cpk mice revealed renal hyperactivation of the glutaminolytic pathway, consistent with a c-Myc role in cellular metabolism (76). The cpk mouse model is also associated with overexpression of Pkd1 in both the cystic kidneys and pancreas (93). More recently, Cystin-1 was shown to interact with Necdin, a DNA binding factor that can activate c-Myc promoter (94). Cystin-1 in a complex with Necdin antagonizes stimulation of c-Myc expression. Importantly, a study on treatment of the cpk mice using c-Myc antisense oligonucleotides led to marked decrease and delay of cyst severity, attenuating PKD significantly (95).

Evidence that c-Myc regulates downstream targets implicated in a cystogenic network was provided by both upregulation and inactivation of miR-17~92. Transgenic mice overexpressing miR-17~92 developed renal tubular and glomerular cysts. Conversely, inactivation of miR-17~92 caused delay of cystic progression in non-orthologous mouse models (96).

Few non-orthologous cystic mouse models known to target Pkd2/PC2 or cilia structure also implicate upregulation of c-Myc. The autosomal dominant mutation in the rat model, Han:SPRD-cy, which is considered a model for human ADPKD, displayed highly elevated levels of renal c-Myc in cystic epithelia from a young age (97). The hepatocyte nuclear factor-1 β (Hnf-1 β), which directly modulates expression of the orthologous Pkd2 gene and the Pkhd1 gene responsible for autosomal recessive PKD, produce upon inactivation a mouse model with diabetes and renal cysts. Notably, kidneys in this mouse model displayed substantial increase in c-Myc expression (96). The motor protein kinesin family 3A, Kif3a, mediates intraflagellar transport in the primary cilium and interacts with PC2 (98). Renal inactivation of Kif3a causes loss of primary cilia and formation of renal tubular cysts (60). This phenotype was associated with elevated c-Myc and β -catenin expression and suggests the activation of the Wnt canonical pathway as detected in human ADPKD (96, 99). The truncated Inversin mouse mutant lacking the C-terminus (Inv Δ C), a mouse model of nephronophthisis type 2, NPHP2, has high levels of c-Myc expression that plays a key role in renal cyst formation (100). Treatment of the Inv Δ C mice with an ERK inhibitor reduced not only the level of phosphorylated ERK but also markedly reduced c-Myc expression associated with decreased proliferation and slower cyst enlargement.

Orthologous PKD mouse models co-associate with c-Myc overexpression

At present, animal models of the two known human genes responsible for ADPKD point to c-Myc as major mediator of cytogenesis *in vivo*. Orthologous dosage-reduced Pkd2 mouse models caused by conditional ablation exhibit increased renal expression of c-Myc (96). Similar to the Pkd2 mouse models, two Pkd1 orthologous dosage-reduced mouse models by conditional Pkd1 ablation, and by Pkd1 hypomorphic mutation, develop renal cysts and also induce elevated c-Myc mRNA and protein (101). The transcriptional upregulation of c-Myc resulted from increased expression of the epigenetic regulator Brd4 that binds to the c-Myc promoter. It was discovered that Brd4 upregulation is caused by the heat shock protein-90 (Hsp90) chaperone activity that protects Brd4 from proteasomal degradation (101), consistent with increased Hsp90 expression in Pkd1 mouse models (102). Most importantly, the use of the BRD inhibitor JQ1 in two Pkd1 mouse models suppressed c-Myc expression and p21 signaling that reduced proliferation and delayed renal cyst growth in both renal deficient and hypomorphic Pkd1 mouse models (101).

Two orthologous dosage-increased Pkd1 mouse models, including one with extrarenal manifestations, reproduce all of the typical PKD characteristics including fibrosis and inflammation (103, 104) (Figure 1). Strikingly, renal analysis in both the Pkd1_{TAG} and SBPkd1_{TAG} mouse models revealed upregulation of c-Myc mRNA expression and protein that is markedly enhanced in the cystic epithelia (104). Pkd1 dysregulation in these mice promotes cellular responses typical of full-length c-Myc. Moreover, the enhanced cilia length quantified in renal epithelial cells of both these Pkd1 dosage-increased models is also consistent with Myc-nick function on α -tubulin acetylation (24, 105). Interestingly, a mouse model that reproduces a naturally-occurring human Pkd1 truncating mutation, Pkd1_{extra}, develops slowly progressive renal cysts with elevated c-Myc expression (106). Notably, overexpression of Pkd2 in transgenic mice leads to increased c-Myc levels in kidneys with cystic anomalies (107, 108).

Molecular PC1 dysregulation identified stimulation of effectors from the Wnt, Hippo, Sonic Hedgehog, Notch, ERK and BMP/TGF β cascades as uncovered by global profiling and candidate approach analyses (74, 75, 96, 109). Remarkably, the signalling pathways activated in the orthologous mouse models virtually mimic those identified in human ADPKD studies, some of which are also deregulated in non-orthologous mouse models and result in c-Myc upregulation. Recently, accumulating evidence has shown that effectors of Wnt and Hippo pathways for example, are often shared and crosstalk through multiple mechanisms (for review (110)). Significantly, targeting several effectors of these pathways led to renal cyst formation in the mouse (111-117). Similarities between dysregulation of polycystin(s) and c-Myc suggest that these proteins are involved in a network or in common signalling pathways essential in renal development and in the ADPKD adult pathologic condition.

In the mouse, c-Myc is not only a target of polycystin(s) but also a critical mediator of cystogenesis. Definitive causal connection between c-Myc and cyst formation was established from the SBM mouse model and from the therapeutic benefit of c-Myc downregulation in the treatment of Pkd1 dosage-dependent mouse models.

Conclusion

Upregulation of c-Myc is considered a typical characteristic hallmark of PKD in the mouse. In fact, one cannot seem to dissociate c-Myc upregulation from renal cystic diseases (Figure 1). The molecular basis for c-Myc activation in PKD1/Pkd1 and PKD2/Pkd2 dysregulation remains incompletely understood. It is most likely that the polycystins PC1 and PC2 influence the activity of c-Myc through interaction within one pathway, or a

network of pathways. Perhaps most significantly, studies abrogating c-Myc expression in particular in orthologous mouse models was therapeutically beneficial with no side effects detected.

On the principle that mouse signalling studies are often validated in humans, c-Myc ought to be a major player in ADPKD signalling from the substantial accumulation of data that has repeatedly placed c-Myc at the centre of the ADPKD scene. The parallel between the characteristic pathologic features of human ADPKD and murine PKD to the typical functions of the c-Myc oncogene is impressive. ADPKD displays numerous cancer-like characteristics, but probably with less severe changes to genome integrity and fewer multistep lesions. The notion that ADPKD appears dependent on the many functions of c-Myc activation at different steps of cyst initiation, progression and expansion suggests the concept of “c-Myc addiction” for cyst development.

Future directions

Although our knowledge of the mechanisms and genes that govern ADPKD has substantially increased over the last years, several outstanding questions remain to be resolved. From a fundamental perspective, it is unclear what molecular mechanisms underlie the fetal-like phenotype in cystic epithelial cells. The immediate downstream effector(s) of polycystin have not yet been determined. One key question is the exact mechanism associated with the integration of the polycystin signalling interactions and networks leading to c-Myc upregulation in PKD. Etiology of the polycystin pathways will provide powerful support to better preventive and therapeutic measures and to the development of treatments. Molecular understanding of the genetic basis and progression mechanisms of PKD will have crucial translational applications for discovery of novel targets to design drug screening. These studies will provide a rational basis for tailored therapy.

From a translational perspective, ADPKD appears to be characterized by cumulative c-Myc-distinct functions that would warrant development of combinatorial therapies. Clearly, multi-targeting various complementary mechanistic functions in ADPKD for clinical therapeutic interventions would counteract a number of external (environmental, epigenetics) and internal (genetic) variations. An integrated ADPKD therapy may not require complete abrogation but moderate inhibition of most c-Myc-distinct functions that could be as efficient and have tremendous impact in a clinical setting with minimized adverse effect.

Conflict of Interest

The author declares that she has no conflict of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 11

The Role of G-protein Coupled Receptor Proteolytic Site (GPS) Cleavage in Polycystin-1 Biogenesis, Trafficking and Function

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Abstract

Polycystin-1 (PC1) is encoded by *PKD1*, the principal gene mutated in autosomal dominant polycystic kidney disease (ADPKD). The protein regulates terminal differentiation of tubular structures in the kidney and is required to maintain their structural integrity. A fundamental property of PC1 is post-translational modification by cis-autoproteolytic cleavage at the G-protein coupled receptor proteolytic site (GPS) motif located at the base

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of the extracellular ectodomain. Defective cleavage likely plays a significant role in the pathogenesis of ADPKD. In mouse models, GPS cleavage of PC1 is essential for the integrity of the distal nephron segments during the postnatal period. While the exact cellular and biochemical functions of PC1 have yet to be fully elucidated, its trafficking and function must be precisely regulated at the subcellular level to ensure proper structure and function of the kidney. Recent evidence shows that GPS cleavage plays a central role in PC1 biogenesis, trafficking and function *in vivo*. GPS cleavage results in the N-terminal fragment (PC1_{NTF}) and C-terminal fragment (PC1_{CTF}), which remain non-covalently associated to form a heterodimeric PC1 molecule. Cleavage is required for ciliary trafficking of PC1, which occurs in a two-step mechanism. First, PC1 interacts with polycystin-2 (PC2) in the ER and binds Rabep1. PC1/2 complex formation is required for transition of PC1 to the trans-Golgi compartment. Second, once arriving at the trans-Golgi, PC1/2-bound Rabep1 recruits GGA1 and the small GTPase Arl3 sequentially for ciliary targeting. In the absence of cleavage, the PC1/2 complex cannot reach the trans-Golgi. This article will discuss the roles of GPS cleavage for PC1 structure, trafficking and function that are relevant for normal activity of polycystin-1 and in cystogenesis of ADPKD.

Key words: Autosomal dominant polycystic kidney disease; Ciliary trafficking; Cystogenesis; GPS cleavage; Polycystin-1

Introduction

Polycystin-1 (PC1) is encoded by *PKD1* (1), the principal gene mutated in autosomal-dominant polycystic kidney disease (ADPKD) (2). Prior work indicates that PC1 regulates signaling pathways essential for proper tubular structures in kidney and liver (3-7) and suggests that a threshold level might be required to prevent cyst formation (8, 9). Cystogenesis will begin when the level of functional PC1 is below the critical threshold (10, 11). This is thought to occur through a “two-hit” mechanism once the cells with inherited heterozygous germline mutations acquire a second, somatic mutation to inactivate the remaining normal allele (12-17). Moreover, the degree to which PC1 activity falls below the threshold will determine disease severity, with lower levels leading to earlier and faster cyst growth. In addition, the level of PC1 is the central determinant of cyst formation in other types of cystic diseases such as autosomal recessive polycystic kidney disease (ARPKD) and autosomal dominant polycystic liver diseases (ADPLD) (18).

PC1 is a 4302-amino acid (aa) 11-transmembrane (TM) receptor-like glycoprotein with a large N-terminal extracellular region (ectodomain) of 3072 residues and a short cytoplasmic C-terminus of ~200 residues (1) (Figure 1). The ectodomain contains a combination of functional domains involved in protein-protein interactions and the ~1000 residue receptor for egg jelly (REJ) module that harbors four bona fide fibronectin III domains (19, 20). Situated at the base of the ectodomain is the ~50-aa G-protein coupled receptor proteolytic site (GPS) motif (21). The cytoplasmic C-terminus is responsible for activating a number of intracellular signaling pathways including the Ca²⁺, cAMP, JAK2/STAT, PI3kinase and mTOR pathways (6, 22, 23). This region contains a coiled-coil domain that binds polycystin-2 (PC2) (24, 25), the *PKD2* gene product (26), which belongs to the transient receptor potential channel family and acts as an ER calcium release channel (27). PC1 is found in a range of subcellular compartments, notably at the plasma membrane and the primary cilium, an organelle that appears most relevant to the pathogenesis of ADPKD (28, 29). PC1 and PC2 are thought to form a receptor-channel complex in cilia (29, 30), with PC1 acting as a sensor of extracellular signals and PC2 as a regulated cation channel. The ciliary polycystin complex is proposed to mediate Ca²⁺-dependent signaling pathways in response to either mechanical or chemical signals through an unknown mechanism (29, 31). While the exact cellular and biochemical functions of PC1 have yet to be fully elucidated, its trafficking and function must be precisely regulated at the subcellular level to ensure proper structure and function of the kidney and other organs.

A fundamental property of PC1 is post-translational modification by cleavage at the juxtamembrane GPS motif (5, 32, 33) (Figure 1). GPS cleavage of PC1 is frequently disrupted in ADPKD (33-36). Disease-associated *PKD1* mutations that disrupt cleavage result in loss of the functional properties of PC1 to activate the JAK2-STAT pathway and induce *in vitro* tubulogenesis of MDCK cells in three-dimensional culture (33). Therefore, defective GPS cleavage of PC1 likely has a significant contribution in the pathogenesis of ADPKD. Prior evidence indicates that GPS cleavage plays a central role in PC1 biogenesis, trafficking and function *in vivo*. This article will discuss the roles of GPS cleavage in these processes, which are central to the normal functional activity of PC1 and to cystogenesis in ADPKD.

Polycystin-1 cleavage at the GPS motif via a cis-autoproteolytic mechanism

PC1 cleavage occurs at the His-Leu-*Thr³⁰⁴⁹ (* indicates scissile bond, with the amino acid number based on human PC1) tripeptide sequence within the GPS motif (37) (Figure 1). PS cleavage takes place in the early stages of the secretory pathway,

presumably within the ER shortly after PC1 synthesis. The cleavage reaction most likely occurs through a *cis*-autoproteolytic mechanism, which is due to a self-catalyzed chemical rearrangement and does not require the intervention of exogenous proteases. The chemical rearrangement is based on the ability of the nucleophilic Thr residue of the tripeptide His-Leu-*Thr to initiate a proximal N-O acyl rearrangement, which converts the peptide (amide) bond to a more reactive ester intermediate (37). The attack of the ester by a second nucleophile, such as a water molecule, leads to the irreversible cleavage of the scissile bond. An analogous mechanism was described for a group of *cis*-autoproteolytic proteins including hedgehog, nucleoporin and glycosylasparaginase, an Ntn hydrolase (38).

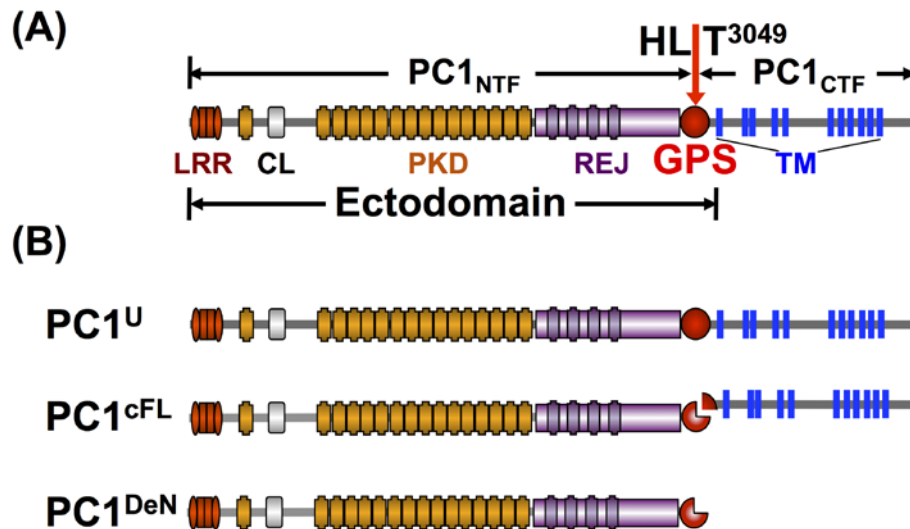


Figure 1. Cleavage of polycystin-1 at the G-protein coupled receptor proteolytic site (GPS) motif. (A) Schematic diagram of the structure of polycystin-1. LRR, leucine-rich repeat; PKD, PKD repeats; CL, C-type lectin; REJ, receptor for egg jelly module with its four fibronectin III domains; GPS, G-protein coupled receptor proteolytic site domain; TM, transmembrane domain. PC1 cleavage occurs at the HL*T³⁰⁴⁹ tripeptide (amino acid numbering based on human PC1) within the GPS motif at the base of the ectodomain, resulting in PC1_{NTF} and PC1_{CTF} fragments as indicated. (B) Polycystin-1 products generated by GPS cleavage. PC1^U, uncleaved full-length PC1; PC1^{cFL}, cleaved full-length PC1 in which PC1_{NTF} and PC1_{CTF} remain non-covalently associated at the GPS; PC1^{DeN}, a separate and stable form of the PC1_{NTF} molecule derived from PC1^{cFL} once it has dissociated from PC1_{CTF}.

GPS cleavage and polycystin-1 molecular complexity

GPS cleavage of PC1 results in formation of an N-terminal fragment (PC1_{NTF}) and a C-terminal fragment (PC1_{CTF}) (33, 37) (Figure 1). A unique outcome of this cleavage is that the two fragments remain tightly and non-covalently associated to form a stable but dissociable heterodimer termed PC1^{cFL} (33, 39). GPS cleavage is generally very extensive in most tissues, with a very small proportion of uncleaved PC1 (PC1^U) molecules detected. In the kidney, cleavage appears to be developmentally regulated. Castelli *et al* (40) recently found that PC1^U is the predominate molecular form in early embryonic kidneys but decreases over time, with the relative amount of cleaved PC1 increasing in a complementary manner. After birth, the proportion of GPS-cleaved PC1 differs between proximal and distal nephron segments (5). While PC1^U remains at a significant level (>50%) in cells of the proximal nephron, most of PC1 is present as the GPS-cleaved molecules (>90%) in distal nephrons.

Pulse-chase experiments showed that GPS cleavage of newly synthesized PC1 can be detected after 15 min of chase, but only about half of the population was cleaved within 2 h, whereas the other half remained uncleaved for a prolonged period of time before finally being degraded (33). Therefore, a significant portion of nascent PC1 molecules appears to be in a cleavage-resistant or blocked state. This notion has led to a model in which newly synthesized PC1 can proceed through two competing pathways: the 'cleavage' pathway, which leads to irreversible cis-autoproteolytic cleavage; and the 'non-cleavage' pathway, which traps PC1^U in the blocked state (37). These data suggested the possibility that cellular factors, including ligand binding, may affect the extent to which PC1 molecules proceed to the cleavage versus the blocked state. It remains to be determined how the differential patterns of GPS-cleaved PC1 observed during kidney development or between different nephron segments are regulated. As discussed in later sections, PC1^U and PC1^{cFL} may have non-redundant functions in different biological processes.

PC1_{NTF} can also be dissociated from PC1_{CTF}, and present as a separate and stable molecule, termed PC1^{deN} (39). In fact, PC1^{deN} exists in significant amounts and can be as much as 10-times the level of PC1^{cFL} in the kidney. The molecular mechanism by which PC1^{deN} is generated is unknown. One possible scenario may involve the differential degradation of PC1_{CTF} due to the PEST sequence (a signal sequence for protein degradation) within the C-terminal tail. Together, GPS cleavage generates a considerable level of complexity of PC1 molecules *in vivo*.

Structural basis of GPS cleavage and subunit association of adhesion GPCRs and the implications for polycystin-1

GPS cleavage is the defining feature of the class of adhesion G-protein coupled receptors (aGPCRs), the second largest subgroup of GPCRs, which are also characterized by long N-termini with multiple functional domains (41, 42). aGPCRs and PC1 have no structural or functional relationship outside of the GPS motif. However, a recent crystallographic study of aGPCRs by Arac et al (34) provided critical insights into both the structural basis of GPS cleavage and the association of cleaved subunits, which have important implications for PC1. The GPS motif forms five β -strands that are tightly integrated into a larger ~320-residue domain termed the GPCR-Autoproteolysis INducing (GAIN) domain that is also present in PC1 (Figure 2).

In the structure of the uncleaved GAIN domain, the scissile bond within the His-Leu-*Thr tripeptide of the GPS motif is positioned at a sharply-kinked loop between the last two β -strands. This distorted and strained geometry favors the equilibrium toward an N-O rearrangement to facilitate ester formation, and thereby provides the necessary driving force for the cis-autoproteolytic reaction. Three structural elements are responsible for keeping the sharp β -turn in place: (1) two disulphide bonds between neighboring β -strands (PC1 has only one disulphide bond, C3015-C3043); (2) an extensive network of hydrophobic interactions between the last β -strand and other residues within the GPS motif; and (3) the trapping of the Leu side chain of the His-Leu-*Thr tripeptide within a conserved hydrophobic pocket. The analogous structural elements are schematically depicted for PC1 in Figure 2.

Following cleavage, the last β -strand remains tightly bound to the remainder of the GPS motif by an extensive network of hydrophobic interactions mediated between side chains. This finding provided the structural basis for the heterodimeric association of GPS cleavage fragments. The overall structural composition of the aGPCR heterodimers is proposed to allow them to participate in different types of cell guidance (34, 43, 44). By analogy, the heterodimeric structure of PC1cFL is likely to be important in enabling the unique biological functions of PC1 within the kidney.

GPS cleavage and polycystin-1 ciliary trafficking

The primary cilium is the key organelle for the control of tubule diameter (45, 46). Defects of cilia lead to ciliopathies, diseases that include cystogenesis in kidneys (47, 48). The ciliary membrane is separated from the plasma membrane by a periciliary diffusion barrier (49).

Ciliary membrane proteins must therefore be transported to the cilium from their site of synthesis in the rough endoplasmic reticulum (ER) for proper ciliary function (50). Experimental evidence favors a targeted delivery model, whereby ciliary membrane proteins are sorted in the Golgi and are then targeted to the cilium by the vesicular pathway via a number of protein complexes such as the BBSome (50) and intraflagellar transport complexes (51).

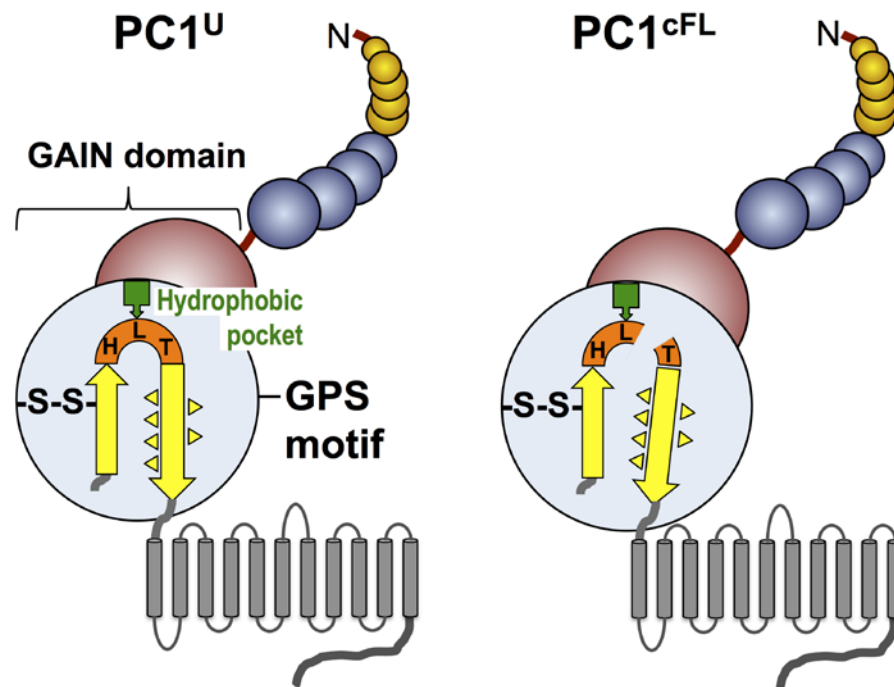


Figure 2. Structural model of the GPS/GAIN domain and heterodimeric association in polycystin-1. The GPS motif in polycystin-1 is schematically depicted based on analogy with the structure of the GAIN domain of the aGPCRs by Arac *et al* (34). In the uncleaved GPS, the scissile bond of the His-Leu-*Thr (HL*T) tripeptide is positioned within a sharply-kinked β -turn between the last two β -strands (the other three β -strands are not shown). This distorted and strained geometry provides the necessary driving force for cis-autoproteolytic cleavage. The structural elements that maintain the sharp β -turn are depicted. In the cleaved GPS, the last β -strand remains tightly bound to the rest of the GPS by the extensive network of hydrophobic interactions as indicated by the triangles. See text for details.

Previous efforts to understand ciliary trafficking of the large transmembrane PC1 and PC2 proteins have focused on the identification of their cilia-targeting motifs and the binding proteins that may mediate their trafficking. For PC1, the ciliary targeting sequence is at its extreme C-terminus (52), while for PC2, the targeting sequence is within the first 15 amino acids at the N-terminus (53). In some studies, PC2 was able to localize to cilia independently of PC1 (53, 54), while other studies show that this requires PC1 (29, 55, 56). In addition, different trafficking routes have been reported for PC1 and PC2 to reach the cilium. PC1 is trafficked to cilia from the trans-Golgi network (TGN) via post-Golgi vesicles in an Arf4-dependent manner (52), whereas PC2 is trafficked to the cilia directly from the cis-Golgi compartment without traversing the Golgi apparatus (54).

Kim *et al* (57) have recently shown that GPS cleavage is required for ciliary trafficking of PC1, which occurs via a two-step mechanism (Figure 3). First, the PC1/2 complex is formed in the ER and binds Rabep1. PC1/2 complex formation is required for transition of PC1 to the trans-Golgi compartment. Second, once arrived at the TGN, PC1/2-bound Rabep1 recruits GGA1 and the small GTPase Arl3 sequentially to enable subsequent ciliary targeting. The following three sections describe these two ciliary trafficking steps for PC1 and discuss the potential roles for GPS cleavage.

Trafficking of polycystin complex to the trans-Golgi network

Kim *et al* (57) have shown that endogenous PC1 and PC2 are mutually required for their ciliary localization in cells and kidney tissues (57). Therefore, the ciliary-targeting signal in each protein appears not to be sufficient for ciliary trafficking in renal epithelial cells. Ectopic expression of PC1 could induce the ER-resident endogenous PC2 to translocate to the cilium. However, amino acid substitutions within the coiled-coil domain of PC1, which disrupt interaction with PC2, abolished the ability of PC1 to traffic, and to induce translocation of PC2, to the primary cilium. PC1 and PC2 must therefore interact to form a molecular complex in order to traffic to cilia. N-glycosylation analyses of native PC1/2 protein complex provided further insights into the role of polycystin complex formation in ciliary trafficking. In the kidney, a significant fraction of the PC1/2 complex was found to be Endo H resistant and characterized by PC2 with a higher molecular weight of ~130 kDa, rather than the predominant cellular PC2 that is Endo H sensitive and has a molecular weight of ~120 kDa. Depletion of PC2 abolished the ability of PC1 to acquire Endo H resistance and to traffic to cilia. These data indicate that PC1-PC2 interaction is required for ciliary trafficking by enabling the polycystin complex to move to the TGN. Given that PC1 is far less abundant than PC2, PC1 is likely the rate-limiting factor for ciliary trafficking of the PC1/2 complex in renal epithelial cells.

Why is the PC1-PC2 interaction required for the complex to move to the TGN? It was previously reported that PC2 is continuously released from the ER to the Golgi in a COPII-dependent manner but immediately returned to the ER via an ER retention/retrieval signal present in its C-terminus (54, 58, 59). The PC1-PC2 interaction may be required to counteract and overcome the retrograde transport of PC2 to the ER, perhaps by masking the ER retention/retrieval signals or alternatively by allowing the complex to pass quality-control checkpoints in the ER as found for many GPCRs (60).

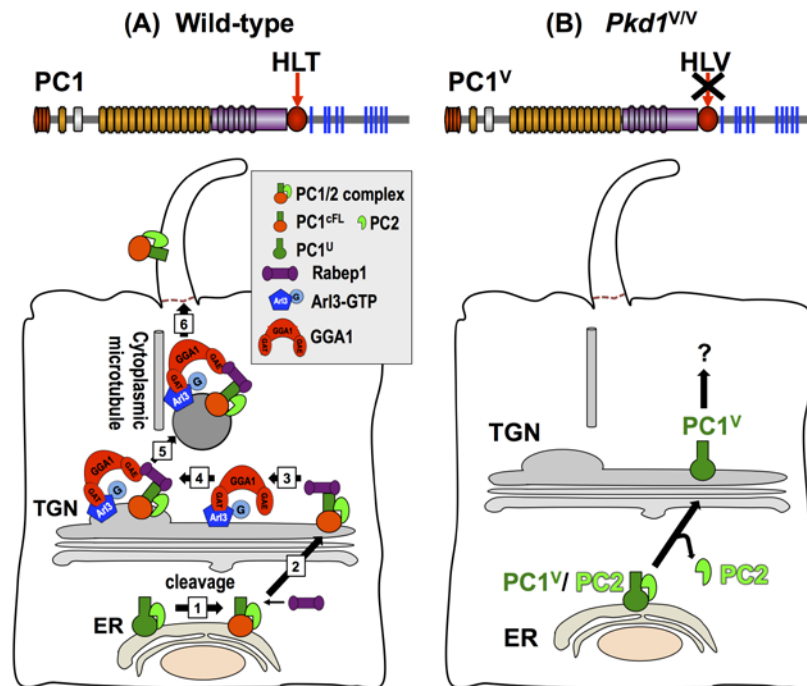


Figure 3. Model for ciliary trafficking of the polycystin complex and the role of GPS cleavage. (A) In wild-type cells, PC1 is cleaved at GPS and forms a complex with PC2 in the ER (1). Rabep1 binds PC1's cytoplasmic C-terminal tail at a pre-Golgi compartment (2), and this complex traffics to the trans-Golgi network (TGN). At the TGN, Rabep1 couples the polycystin complex to a GGA1/Arl3 module (3), which is formed by Arl3-GTP binding to GGA1. GGA1 then assembles the clathrin coat (4) to form the vesicle carrier (5). The resulting polycystin complex-bearing vesicle traffics along the cytoplasmic microtubules to the base of cilia (6) and enters the cilium by an unknown mechanism. (B) In the absence of GPS cleavage as in *Pkd1^{V/V}* mice, PC1V is able to interact with PC2 to form a PC1V/PC2 complex in the ER. However, PC1V cannot maintain a stable association with PC2 at the trans-Golgi, nor traffic to cilia. The final subcellular location of PC1V remains to be determined. The figure is adapted from "Ciliary membrane proteins traffic through the Golgi via a Rabep1/GGA1/Arl3-dependent mechanism" by Kim et al., Nat Commun. 2014 Nov 18;5:5482. doi: 10.1038/ncomms6482 (57).

Rabep1/GGA1/Arl3-dependent ciliary trafficking of polycystin complex

N-glycosylation analyses of intact, isolated ciliary preparations showed that cilia contain only Endo H-resistant forms of PC1 and PC2 (57). This result provides direct biochemical evidence that the ciliary polycystins are derived from the TGN. How is the polycystin complex at the trans-Golgi directed to cilia?

We recently published data demonstrating that a novel protein complex composed of Rabep1, GGA1 and Arl3 is critically involved in mediating the sorting and targeting of the polycystin complex from the TGN to the cilium (57) (Figure 3). Using the yeast two-hybrid system, we identified Rabep1 as a binding partner of PC1 at its C-terminus. Rabep1 is an effector of multiple Rab GTPases involved in various steps of intracellular vesicular trafficking but not previously known for ciliary trafficking (61). Rabep1 knockdown in collecting duct cells abolished the ciliary localization of PC1 and PC2. Rabep1 binds the polycystin complex and thereby accompanies it to the Golgi. Once arrived at the TGN, PC1/2-bound Rabep1 binds Golgi-localized GGA1 (Golgi-localized, gamma adaptin ear-containing, ARF-binding), which is known to mediate the ARF-dependent recruitment of clathrin to the TGN (62-64). Rabep1-GGA1 interaction was previously described in the context of the fusion of TGN-derived vesicles with endosomes (65). GGA1 knockdown in collecting duct cells abolished the ciliary localization of PC1 and PC2. Arf4 was previously proposed to be involved for ciliary trafficking of a PC1 C-terminal fragment (52). However, Arf4 was not detected in the native polycystin complex and therefore does not appear to play a significant role in its ciliary trafficking (57). Instead we found association of a closely related member of the Arf family, Arl3, with the polycystin complex. Moreover, Arl3 knockdown in collecting duct cells abolished the ciliary localization of PC1 and PC2, indicating a critical role for Arl3 in the ciliary targeting of the polycystin complex. In mice, Arl3 inactivation was previously shown to result in renal cystogenesis (66) that is similar to the *Pkd1^{V/V}* knockin mutant mice (5) (see following sections). Collectively, our data support a model in which Rabep1 recruits the polycystin complex to GGA1/Arl3 at TGN for ciliary trafficking.

The molecular mechanism by which Arl3 regulates ciliary trafficking of polycystin complex is unclear. Arl3 is exclusively found in ciliated organisms (67), and localizes to a number of microtubule-dense structures including the centrosome and cilia, as well as on the Golgi (68). It has microtubule-binding activity and is suggested to function in microtubule-dependent processes in mammalian cells (68). In fact, Arl3 is described to regulate vesicle transport from the Golgi to pericentrioles in photoreceptors (69). One possibility is therefore that Arl3 might direct polycystin-bearing vesicles to cytoplasmic microtubules for cytoplasmic dynein-driven transport to the cilium. Cytoplasmic dynein (distinct from ciliary dynein) is a multi-subunit molecular motor that drives microtubule-based

retrograde vesicle transport toward minus-ends (70). It is reported to drive transport of rhodopsin-bearing vesicles from Golgi to the connecting cilium in photoreceptors (71). Recent evidence also indicated a role for Arl3 in releasing lipid-modified proteins from their soluble transport proteins inside cilia (72, 73). It remains to be determined whether Arl3 has a similar role for the polycystin complex once arrived inside the cilium.

Role of GPS cleavage in ciliary trafficking of polycystin complex

Analyses of intact ciliary preparations isolated from renal epithelial monolayers expressing recombinant PC1 showed that cilia contain only cleaved PC1 (57). The uncleaved PC1^U was absent from the cilia, although it was present at levels similar to cleaved PC1 within the cell body. Two possible mechanisms could be responsible for the lack of PC1^U in cilia. First, PC1 has to be cleaved in order to traffic to cilia. Second, PC1^U can traffic to cilia but becomes rapidly cleaved before reaching the cilia. To differentiate between these two possibilities, a non-cleavable PC1 mutant termed PC1^V, with a Thr-to-Val substitution at the His-Leu*Thr cleavage site was engineered and analyzed (37, 39) (Figure 3). Thr and Val differ solely by one functional group at the very terminus of the side chain (-OH vs. -CH₃). While effectively preventing cleavage by preventing the nucleophile attack, the critical initial step of cis-autoproteolysis (37), this smallest possible change seems less likely to significantly alter the conformation surrounding the cleavage site. The equivalent mutation did not cause conformational changes in the cis-autoproteolytic protein as shown by structural analysis (74). It is reasonable to assume that this mutation only blocks cleavage, without major unintended “side effects” that may confound the interpretation of the results. In fact, PC1^V did not traffic to cilia, nor did it induce the ciliary translocation of endogenous PC2. These findings indicate a critical role for GPS cleavage in ciliary trafficking of polycystin complex, rather than reflect efficient cleavage of PC1 prior reaching the cilia.

N-glycosylation analyses with *Pkd1^{V/V}* knockin mice, which harbor the same Thr-to-Val substitution and thus express mutant PC1^V, provided critical insights into the role of GPS cleavage in ciliary trafficking of the polycystin complex (39, 57). We found that PC1^V acquires Endo H resistance to a significant degree (~50%) in *Pkd1^{V/V}* tissues and cells. This finding indicates that GPS cleavage is not a prerequisite for trafficking of PC1 to the Golgi compartment. PC1^V retains its ability to interact with PC2 and forms a PC1^V/PC2 complex in the ER (Figure 3). However, PC2 in this complex remains entirely sensitive to Endo H. These results indicate that GPS cleavage is not necessary for the formation of the PC1/2 complex, but is required to enable the complex to traffic to the trans-Golgi compartment. In the absence of cleavage, the PC1^V/PC2 complex cannot reach the trans-Golgi; instead, PC1^V arrives at the Golgi without the association of PC2. Therefore, GPS cleavage may be required to prevent premature dissociation of the PC1/2 complex. One possibility is that

cleavage increases the affinity of PC1-PC2 binding and thereby ensures stable association of the complex at the Golgi. An alternative, but not mutually exclusive, idea is that GPS cleavage may increase the ER-to-Golgi transportation rate of the polycystin complex, thereby outpacing PC2 dissociation during the transition. It would be of interest to determine whether GPS cleavage may also play a critical role in recruiting Rabep1, GGA1 or Arl3, and/or whether PC2 is important for this process.

Critical and restricted functional role of PC1 cleavage at GPS *in vivo*

Important insights into the functional role of GPS cleavage were gained by the characterization of the *Pkd1*^{V/V} knockin mouse (Figure 4), the first *Pkd1* mouse model with a missense mutation. PC1^V is present at two to three times the level of wild-type PC1^U, with similar stage- and tissue-specific expression patterns (5). The mutant mice exhibit several important phenotypic differences compared to the *Pkd1* knockout mice. While *Pkd1* knockout mice develop massive renal and pancreatic cysts during embryonic development and are embryonically lethal (3, 4), *Pkd1*^{V/V} mice are viable with apparently intact kidney and pancreas structure at birth. However, the *Pkd1*^{V/V} mutants develop rapid cystic dilation of collecting ducts (except the papilla tip) and distal convoluted tubules starting at postnatal day 3 (Figure 4).

Unexpectedly, *Pkd1*^{V/V} mice do not display cystogenesis in the proximal tubules, thick ascending limb of loop of Henle, or the pancreas during their lifespan of up to 3 weeks. Therefore, cleavage is dispensable for the embryonic development of various organ systems, including kidney and pancreas, and for proximal nephron segments after birth, but is essential for the integrity of the distal nephron segments during the postnatal period. Collectively, GPS cleavage plays a critical and restricted role in PC1 function *in vivo*.

The importance of GPS cleavage was recently confirmed by the *BAC-Pkd1* transgenic approach using a different non-cleavable mutant, PC1-L3040H, which contains a Leu-to-His substitution at the penultimate position of the His-Leu-*Thr cleavage site (35). Unlike PC1^V in the *Pkd1*^{V/V} knockin mice, PC1-L3040H did not rescue the embryonic lethality of *Pkd1*^{-/-} mice, indicating a complete loss-of-function by this mutant. Remarkably, in contrast to PC1^V, PC1-L3040H did not acquire Endo H resistance, which is indicative of a trafficking defect. Based on the structural analysis of the aGPCR GPS/GAIN domain (34), Leu3040, which is highly conserved in all GPS motifs, is expected to form part of the hydrophobic pocket at the sharp kink of scissile bond (see Figure 2). Substitution with the bulky and charged His at this position may therefore alter the conformation of the GPS/GAIN domain preventing cleavage and secondarily disrupting exit from the ER, thereby confounding the role of GPS cleavage. These considerations highlight the importance of experimental design for assessing the function of GPS cleavage.

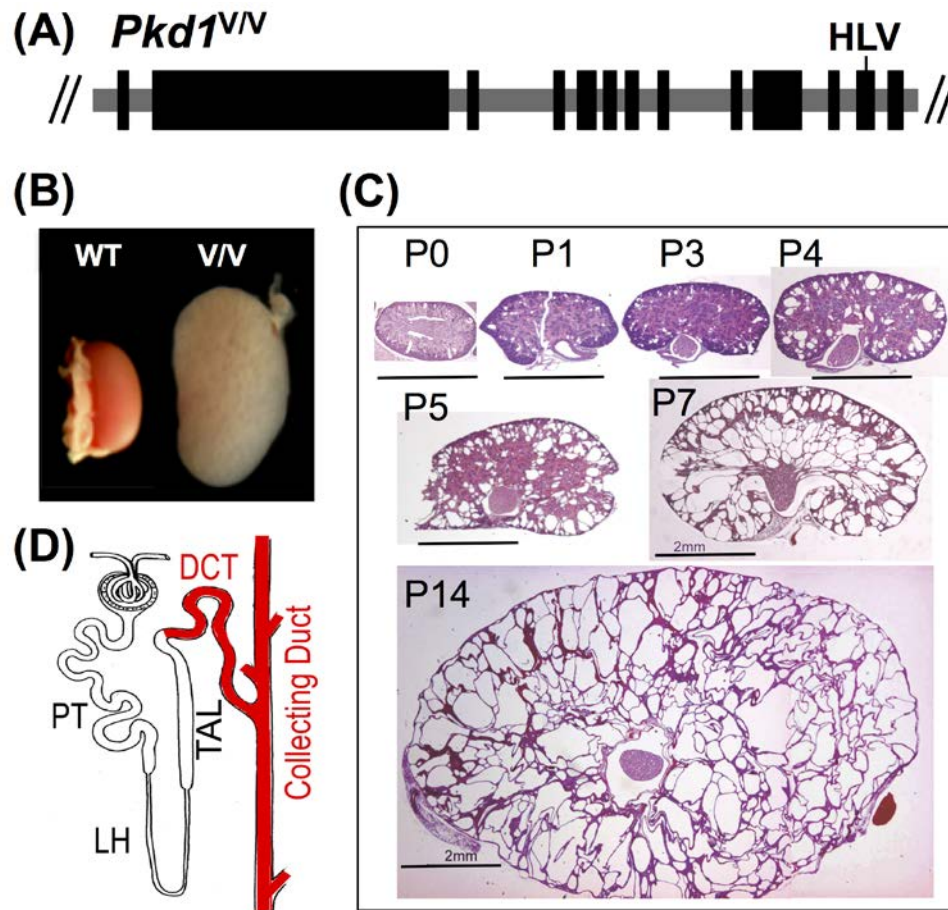


Figure 4. Essential biological function of polycystin-1 cleavage within the GPS motif. (A) Diagram of the *Pkd1*^{V/V} knock-in allele. Exons are depicted by black boxes. The critical nucleophilic threonine residue at the HLT cleavage site (in exon 25 of the *Pkd1* gene) is mutated to a non-polar valine by homologous recombination. (B) The *Pkd1*^{V/V} kidneys (V/V) are enlarged, pale, and cystic compared with normal kidneys from wild-type (WT) littermates as shown for postnatal day 9. (C) Hematoxylin and eosin-stained sections of *Pkd1*^{V/V} kidneys at various postnatal stages, demonstrate the rapid and progressive cystic dilation of *Pkd1*^{V/V} kidneys during the postnatal period. Scale bars, 2 mm. (D) Diagram of the nephron segments affected by cystogenesis in *Pkd1*^{V/V} kidneys. Cysts are derived from distal convoluted tubule (DCT) and collecting duct (in red). Glomerulus, proximal tubule (PT), loop of Henle (LH), and thick ascending limb (TAL) are not dilated. The figure is adapted from "Essential role of cleavage of Polycystin-1 at G protein-coupled receptor proteolytic site for kidney tubular structure" by Yu *et al.*, Proceedings of the National Academy of Sciences of the United States of America 2007, 104:18688-18693 (5).

Do PC1^U and PC1^{cFL} have different biological functions?

What is the molecular mechanism by which GPS cleavage affects PC1 function? In principle, GPS cleavage may result in a more active PC1 molecule, for example by the creation of a high-affinity binding pocket for as-yet unidentified ligands, as has been suggested for other receptor molecules, or by more efficient signal transduction after ligand binding (75). Given the distinct patterns of GPS cleavage of PC1 during kidney development and in different nephron segments, and the critical role of cleavage in ciliary trafficking, it is tempting to speculate that PC1^U and PC1^{cFL} have non-redundant functions in different biological processes.

In early embryonic stages, PC1 exists largely in PC1^U form. Therefore, PC1^U might mainly traffic, perhaps apart from PC2, to a non-ciliary location such as cell-cell junctions where it could regulate convergent extension and elongation of developing renal tubules (40, 76). After birth, however, most of the PC1 in distal tubules and collecting ducts is GPS cleaved (5, 39), and PC1^{cFL} traffics to the cilia in the form of the PC1/2 complex to control their proper tubular diameter. On the other hand, GPS cleavage is not required for intact proximal tubules in which PC1^U is the more abundant molecule (5). PC1^U may play a key role in the integrity of this portion of the nephron. The subcellular trafficking and biological functions of PC1^U and PC1^{cFL} in the proximal tubules remain to be examined. PC1^{deN} itself does not have an intracellular region and its function is currently unknown (39). In summary, GPS cleavage may be significant for regulating polycystin trafficking and function in the kidney in a development- or nephron segment-specific manner.

GPS cleavage of polycystin-1 and polycystic kidney disease

GPS cleavage of PC1 appears to be frequently disrupted in human ADPKD. Of the 15 ADPKD-associated missense mutations in the GPS motif and the adjacent REJ module that have been analyzed thus far, all have been shown to disrupt cleavage (33-36). Remarkably, 94 (30%) of the 311 *PKD1* missense mutations classified as pathogenic in the Mayo PKD Mutation Database (<http://pkdb.mayo.edu/>) are located in the REJ-GPS region. By extrapolation, as much as 30% of the pathogenic missense mutations in *PKD1* have the potential to affect PC1 cleavage. These mutations might affect the critical residues that are involved in establishing the strained geometry critical for the cleavage reaction. Because *cis*-autoproteolysis depends on the correct alignment of critical residues within the GPS/GAIN domain, and thus correct global protein conformation (77), *PKD1* mutations that are distant from the GPS and REJ module might also affect cleavage. On the other hand, genetic modifiers or environmental factors that affect protein folding and maturation

have been reported to affect GPS cleavage and thereby disease progression (18, 78). Therefore, defective GPS cleavage of PC1 may play a significant role in the pathogenesis of ADPKD. Disease-associated *PKD1* mutations that disrupt cleavage may result in defects of intracellular trafficking and loss of the functional properties of PC1 preferentially within distal nephron segments.

Conclusion

GPS cleavage is the central control mechanism of normal PC1 biogenesis, trafficking and function, as well as a significant factor in ADPKD pathogenesis. Future studies will be directed to elucidate molecular mechanisms by which cleavage and a heterodimeric structure allow the PC1 molecule to mediate signal transduction at cilia and possibly other subcellular locations, and to develop strategies to manipulate GPS cleavage or the extent of heterodimeric association of PC1. These studies are expected to result in novel therapies to target ADPKD.

Conflict of interest

The author declares that he has no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 12

Epigenetics in ADPKD: Understanding Mechanisms and Discovering Treatment

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Abstract

Epigenetics is the study of all heritable changes in gene expression and chromatin organization that are caused by mechanisms independent of the DNA sequence itself. Similar to the genetic information found within the sequence of DNA, epigenetic information can also be inherited across generations. Epigenetic gene regulation includes, but is not limited to, DNA methylation and histone modification through acetylation, methylation, ubiquitylation, phosphorylation, or sumoylation. The roles of epigenetic modulation on gene expression and protein function have recently become the focus in autosomal dominant polycystic kidney disease (ADPKD). An interactive picture between

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PKD gene mutations and the epigenome needs to be developed to understand why inherited PKD gene mutations in patients may result in epigenetic changes that increase the progression of renal cyst formation. Recent studies demonstrate that PKD1 mutation increases the expression of epigenetic regulators, including DNA methyltransferases (DNMTs), histone deacetylases (HDACs), histone methyltransferases (HMTs) and bromodomain proteins. Conversely, inhibition of epigenetic regulators delays cyst growth in *Pkd1* knockout mouse models, supporting the importance of abnormal epigenetic regulation in ADPKD. One of the exciting findings is that targeting Sirt1, a class III HDAC, with nicotinamide (vitamin B3) delays renal cyst growth and preserves renal function in three *Pkd1* knockout animal models. The hypermethylation of PKD1 gene in gene-body regions implicates that DNA methylation-mediated epigenetic silencing of PKD genes is also a potential mechanism underlying cystogenesis. In this chapter, we will summarize the current knowledge on the role of epigenetics in ADPKD and its translational potential to identify much needed new therapies. We will also discuss the tools to study epigenetic mechanisms in ADPKD and their applications on understanding how epigenetic events intertwine with PKD-associated signaling pathways, including c-Myc, EGFR, HSP90, STAT3/STAT6, AMPK, Wnt/ β -catenin, ILK/mTOR, hedgehog, GSK3 β and NF- κ B/inflammation signaling.

Keywords: Epigenetics; DNA methylation; Histone modification; PKD associated signaling pathways; Vitamin B3

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common life-threatening genetic disorders and affects approximately 600,000-700,000 people in the United States (1). The hallmark of the disease is the development of fluid-filled cysts in the nephrons of both kidneys, resulting in end-stage kidney disease (ESKD) and requiring painful dialysis. ADPKD are caused by mutations in either PKD1 gene encoding polycystin-1 (PC1), accounting for 85-95% of the cases, or PKD2 gene encoding polycystin-2 (PC2), accounting for the remainder (2). PC1 can form a complex with PC2 which is a calcium-permeable cation channel (3-6). Renal cyst formation can be initiated at all stages of kidney development, which is also associated with renal interstitial inflammation and fibrosis (7, 8). ADPKD patients also develop various extra-renal manifestations including hepatic cysts, intracranial aneurysms and cardiac vascular abnormalities. In addition to the necessity to be finely tuned to the expression of polycystins (9, 10), multiple signaling pathways downstream of PKD gene mutations have been identified in regulating cystic renal epithelial cell proliferation and apoptosis, leading to cyst formation (11-20).

Elucidation of the complex pathways that regulate the expression of polycystins or the signaling pathways downstream of polycystin signaling are critical for achieving a full understanding of ADPKD pathogenesis and for identification of crucial regulatory or structural components that may be useful as therapeutic targets. Apart from the genetic traits of ADPKD, the roles of epigenetics have recently drawn attention of scientific investigation in ADPKD (21-26). This chapter summarizes the current knowledge on the role of epigenetics in ADPKD, and its translational potential to identify much needed new therapies.

The epigenome and mechanisms of epigenetic regulation

In mammals, genomic DNA is spun around histone protein cores containing dimers of histones H2A, H2B, H3 and H4 to form chromatin (27). Epigenetic modifications on histone proteins and the DNA wrapped around them result in either loose (euchromatin) or tight (heterochromatin) states of chromatin. Euchromatin allows RNA polymerases and transcriptional factors to bind whereas heterochromatin is associated with transcriptional inactivation (27). Epigenetic marks including DNA methylation, histone post-translational modifications, and noncoding RNAs collectively form the 'epigenome'. The close association between DNA methylation and histone modification is well established (28). Perturbations in the epigenome have been implicated in various pathological conditions including cancer and ADPKD (22, 25, 29).

DNA methylation as the first identified epigenetic modification has been intensively studied for half a century (30). DNA methylation is catalyzed by a family of DNA methyltransferases (DNMTs) which can post-replicatively add methyl groups to the C5 position of cytosines in DNA (31). DNA methylation is usually associated with transcriptional silencing of a number of genes and sequence classes, including tumor suppressor genes, imprinted genes, and genes on the inactive X chromosome (32). Silencing of these sequences is essential for maintaining chromosome stability. DNA methylation is distributed throughout the genome generally at CpG dinucleotides, a cluster of large repetitive sequences (called CpG islands) in regions such as centromeric repeats or at the 5' ends of many genes (33). In humans, 50-70% of all CpGs are methylated, primarily in heterochromatic regions. In vertebrates, there are five known DNMTs with different structure and function. All DNMTs, except DNMT2, have an N-terminal regulatory domain and a C-terminal catalytic domain. The ubiquitously-expressed DNMT1, which displays a strong preference for hemimethylated CpG sites, functions to maintain the DNA methylation patterns established by the DNMT3 subfamily, comprising DNMT3a and DNMT3b, on unmethylated DNA (31, 34, 35)

during DNA replication and DNA repair (36, 37). The cofactor DNMT3L1 stimulates the activity of DNMT3a and DNMT3b (38), but by itself lacks enzymatic activity (39). The fifth member of the DNMT family, DNMT2, has very weak activity toward DNA (40, 41). Promoter DNA methylation is a relatively stable epigenetic modification which represses transcription via interference with transcription factor binding or recruiting repressor complexes consisting of methyl-DNA binding proteins (42), such as methyl-CpG-binding domain proteins (MBDs), UHRF proteins (ubiquitin-like, containing plant homeo domain [PHD] and really interesting new gene (RING) finger domains) and zinc finger proteins (43). DNA demethylation occurs mainly by passive mechanisms during development and cell division (44, 45). Aberrant expression of DNMTs and disruption of DNA methylation patterns are closely associated with many forms of cancer. In general, hypermethylation occurs on tumor suppressor genes and hypomethylation occurs on oncogenes (46-48), although the exact mechanisms underlying this link remain elusive.

Histone post-translational modifications as epigenetic marks, including histone lysine acetylation (HKAc), methylation (HKme) and phosphorylation (49), regulate chromatin structure and gene expression (50, 51). Histone acetylation is mediated by histone acetyl transferases (HATs) and is generally associated with relaxed chromatin and active gene expression. In contrast, histone deacetylation is mediated by histone deacetylases (HDACs) and is generally associated with closed chromatin and represses gene expression. On the other hand, histone/lysine methylation is mediated by histone methyltransferases (HMTs) (52) and can be an active or repressive mark depending on the lysine residue modified and the extent of methylation (mono-, di-, or tri-) on different lysine residues. Methylation of lysine residues on histone tails can be erased by histone demethylases (53). Histone modifications can mark and define distinct regulatory regions of the genome, which can serve as docking sites for coactivators, co-repressors, chromatin remodeling proteins, and proteins that bind to modified histones (50, 51, 54). For example, bromodomain proteins, which have an approximately 110 amino acid protein domain called bromodomain, recognize and bind monoacetylated lysine residues on the N-terminal tails of histones (55), whereas chromodomain protein, which has an approximately 40-50 amino acid protein domain called chromodomain (*chromatin organization modifier*), only recognizes and binds methylated histones (56, 57) and appear in the RNA-induced transcriptional silencing complex (58). In general, trimethylation of H3 at lysine 27, namely H3K27me3, is a strong repressor of transcription by attracting chromodomain-containing proteins and HP1 (59, 60).

Noncoding RNAs, including short microRNAs (about 22 nucleotides in length) and long noncoding RNAs (4200 nucleotides long), are also epigenetic marks which work via

epigenetic mechanisms (61-64). The roles of several microRNAs in renal disorders including PKD has recently been studied (65, 66) and will be discussed in next chapter.

The progression of epigenetic studies in ADPKD

DNA methylation and ADPKD

Aberrant DNA hypermethylation and hypomethylation patterns have been associated with human cancer and other diseases (67) and may play a role in the manifestation, progression and therapy of PKD. It has been reported that *PKD1* is hypermethylated in gene-body regions, and its expression is downregulated in ADPKD (Figure 1), implicating DNA methylation-mediated epigenetic silencing as one of the mechanisms underlying cystogenesis (68). Whether the methylations of the *PKD2* gene and autosomal recessive PKD (ARPKD) genes, as well as the genes of PKD-associated signaling pathways, are changed and contribute to cyst development needs be investigated. In addition, *PKD1* mutations result in the upregulation of DNA methyltransferase 1 (DNMT1) in cystic renal epithelial cells (unpublished data); thus genes downstream of PKD mutations (including ADPKD and ARPKD genes) may also be hypermethylated during cyst formation. Further studies should focus on when and how DNA methylation is altered during cyst development, and whether reversal of DNA methylation variations in the early stages of PKD can delay cyst growth and the progression to ESKD.

Histone deacetylases (HDACs) in ADPKD

Evidence generated to date indicates that HDACs are important regulators of ADPKD (21-26). Depending on the sequence similarity and cofactor interactions, HDACs are classified into four classes: Class I HDACs (HDAC1, 2, 3 and 8), which are nuclear enzymes and can be widely expressed in different tissue types (69); Class II HDACs (HDAC4, 5, 6, 7, 9 and 10) and class IV HDAC (HDAC11), which are predominantly located within the cytoplasm and can be expressed in a tissue-specific manner (69); and Class III HDACs, which are called sirtuin family proteins (SIRT1-8) with different subcellular localizations, substrate specificities and functions (70). HDACs are able to deacetylate histones or non-histone substrates, for example, transcriptional factors, to either regulate the expression of the *PKD1* gene or genes and proteins involved in regulating cystic renal epithelial cell proliferation and apoptosis (Figures 1 and 2) (11, 21). Pharmacological inhibition of HDACs delays cyst growth and preserve renal function in *Pkd1* (21-26) and *Pkd2* mutant mice (71), respectively, implicating the potential clinical application of HDAC inhibitors on ADPKD treatment.

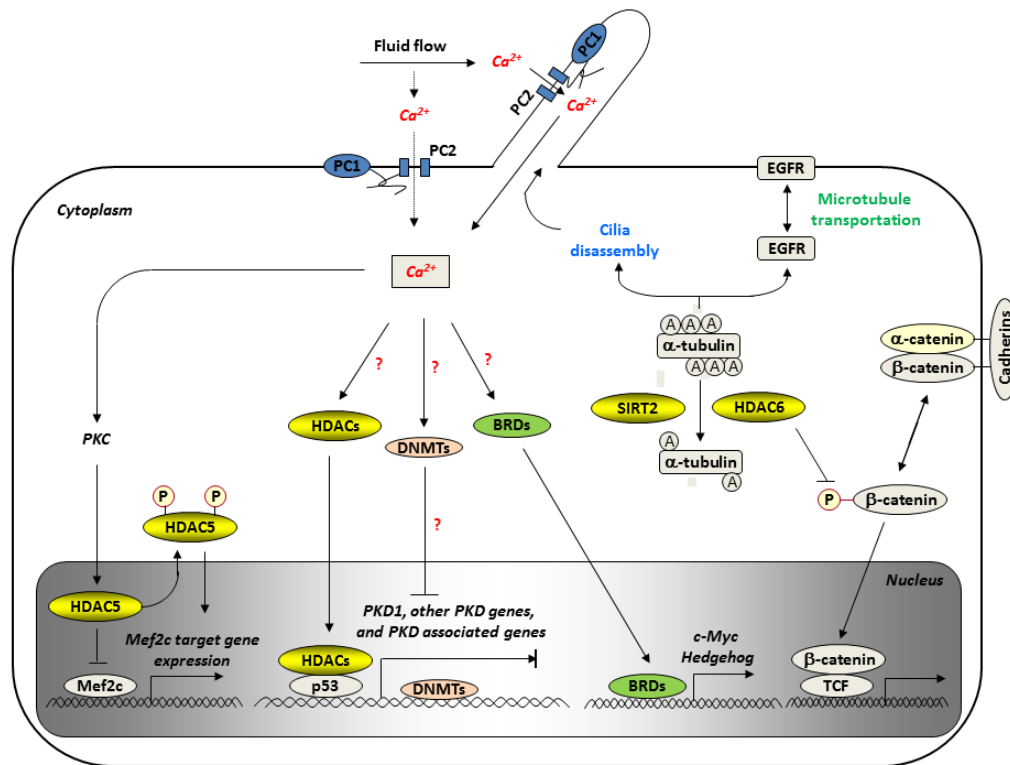


Figure 1. The roles of histone deacetylases (HDACs), Bromodomain proteins (BRDs) and DNA methyl transferases (DNMTs) in renal epithelial cells. In this schematic diagram, we depicted the roles of HDACs in regulating PKD1 gene expression and PKD associated signaling, including that i) HDAC5 is the target of fluid flow-induced calcium signal in renal epithelial cells; ii) HDAC6 and SIRT2 regulate cilia disassembly through deacetylation of α -tubulin during the normal cell cycle; iii) HDAC6 regulates epidermal growth factor receptor (EGFR) trafficking through deacetylation of α -tubulin; and iv) HDAC6 either alone or with EGF regulates β -catenin nuclear translocation. We also indicate the potential roles of DNMTs in regulating the transcription of PKD1 gene, other PKD genes and PKD-associated genes. The roles of BRDs in regulating the transcription of c-Myc and the components of Hedgehog signaling are also included. The involvement of calcium signaling in these processes is possible but is uncertain.

HDACs are involved in repression of the expression of PKD1 gene

The expression of polycystins is required to be finely tuned to prevent cyst formation (9, 10). The PKD1 gene promoter contains a hybrid p53-Sp1-binding motif which has been shown to be bound by p53 *in vivo*. Binding of p53 to the promoter of PKD1 gene decreases

its expression. This process is also regulated by HDACs since a pan-HDACs inhibitor trichostatin A (TSA) could attenuate p53-induced repression of the PKD1 expression (72). Although we propose a model that polycystin signaling activates p53 (73), which in turn, in cooperation with HDACs, controls PKD1 gene expression (Figure 1), however, the role of p53 in regulating mutant PKD1 gene expression needs be further investigated. In addition, the HDAC(s) involved in p53-mediated repression of PKD1 gene is unknown. HDAC1 interacts with p53 and Sp1, which suggests that it may be involved in p53-mediated repression of PKD1 through deacetylation of p53 (74, 75).

HDAC5 is the target of fluid flow-induced calcium signal in renal epithelial cells

HDAC5, a class II HDAC, was identified as one of the targets of polycystin-dependent fluid stress sensing in renal epithelial cells by microarray analysis (76). Fluid flow stimulation of polarized renal epithelial monolayers results in calcium influx into the cells to activate protein kinase C (PKC). PKC then directly or indirectly phosphorylates HDAC5 at two 14-3-3 binding sites, leading to the translocation of HDAC5 from the nucleus to the cytosol (Figure 1) (77). Nuclear export of HDAC5 releases its inhibition on MEF2C-based transcription (78, 79). Heterozygous knockout of HDAC5 or inhibition of HDAC5 activity with TSA delayed cyst growth in *Pkd2*^{-/-} mouse embryos, a result that supports an epistatic relationship between *Pkd2* and HDAC5 (76). In addition, treatment with TSA also delayed cyst growth in kidneys from *Pkd1*^{-/-} embryonic mice (23). Furthermore, treatment with valproic acid (VPA), a class I HDAC specific inhibitor, slowed cyst growth and the decline of kidney function in *Pkd1* conditional knockout mice (71). These results suggest that class I and II HDACs are the potential therapeutic targets for the treatment of ADPKD.

HDAC6 regulates epidermal growth factor receptor (EGFR) endocytic trafficking and degradation as well as β -catenin nuclear localization in renal epithelial cells

HDAC6, a microtubule-associated α -tubulin deacetylase, demonstrates increased expression and activity in *Pkd1* mutant renal epithelial cells (24). The epidermal growth factor-EGF receptor (EGF-EGFR) axis has a documented role in the expansion of renal cysts (80). Inhibition of HDAC6 with TSA or tubacin, a specific HDAC6 inhibitor, increased α -tubulin acetylation and decreased the expression of EGFR in *Pkd1* mutant renal epithelial cells. HDAC6, through deacetylation of α -tubulin, affects the stability of microtubule, which further regulates EGFR intracellular trafficking and degradation along microtubules in normal and mutant renal epithelial cells (Figure 1) for the following reasons: 1) targeting HDAC6 with pharmacological inhibitor not only increased EGFR endocytic trafficking but also normalized the localization of EGFR from apical to basolateral of the cystic epithelial cells in *Pkd1* conditional knockout mouse

kidneys; and 2) treatment with nocodazole, which depolymerized microtubules, decreased the degradation of EGFR and EGFR endocytic trafficking from early endosomes to later endosomes in *Pkd1* mutant renal epithelial cells stimulated with EGF. In addition, HDAC6 is through deacetylation of β -catenin at lysine 49, a site often mutated in cancers, to increase the nuclear localization of β -catenin induced by EGF (81, 82). Inhibition of HDAC6 not only blocks EGF-induced β -catenin nuclear localization but also decreases c-Myc expression, leading to decrease epithelial cell proliferation. In addition, HDAC6 forms complex with HSP90 and MIF (83, 84), the two recent identified PKD associated signaling (7, 11). These results suggest targeting HDAC6 may be a potential therapeutic approach for polycystic kidney disease.

SIRT1 regulates cyst development through deacetylation of Rb and p53 in ADPKD

SIRT1, a member of class III HDACs, targets both histone and nonhistone proteins. SIRT1-mediated histone deacetylation, including histones H1K26, H3K9 and H4K16, is necessary to form heterochromatin and to silence the transcription (85). SIRT1 also deacetylates non-histone proteins, including the retinoblastoma (Rb) protein, E2F1, p53, nuclear factor-kappaB (NF- κ B), FOXO1, FOXO3, c-Myc, β -catenin, heat shock protein 90 (HSP90), and Smad7 to potentially regulate cell proliferation and apoptosis (86-89). SIRT1 can remove an acetyl group from acetylated lysine residues of histone and non-histone proteins to generate lysine, 2'-O-acetyl-ADP-ribose (OAADPr), and nicotinamide which is also a noncompetitive inhibitor of SIRT1 (90, 91).

SIRT1 was upregulated in embryonic and postnatal *Pkd1* mutant mouse renal epithelial cells and tissues, partially through c-Myc and tumor necrosis factor- α (TNF- α) signaling (Figure 2) (22). SIRT1 deletion delayed cyst formation and normalized kidney function in a *Pkd1* mutant mouse model (22). SIRT1 regulates cystic renal epithelial cell proliferation and apoptosis through deacetylation and increased phosphorylation of Rb (at residue S780) which becomes inactive, in turn enabling transcription of genes that mediate entry into the S-phase of the cell cycle (89) and through deacetylation of p53 (22), an important tumor suppressor protein, at residue K382, respectively. Inhibition of SIRT1 with nicotinamide or EX527, a specific SIRT1 inhibitor, decreased proliferation and increased apoptosis of cystic epithelial cells. Targeting SIRT1 with nicotinamide delayed cyst growth, decreased kidney weight to body weight (KW/BW) ratio and decreased blood urea nitrogen (BUN) levels in *Pkd1* knockout mouse models (22). This study provides strong evidence that nicotinamide is a particularly attractive candidate for treatment of PKD.

Nicotinamide (also known as niacinamide) is a water-soluble amide derivative of nicotinic acid, which represents a major form of vitamin B3. Nicotinamide has been

studied in humans for several decades and has an excellent safety profile when given at adult doses of no more than 3 g/day (92). Nicotinamide has been used in humans at doses ranging from 1 g/m² to 6 g daily as an anti-oxidant, anti-inflammatory and immune modulator in many disease conditions, including type I diabetes mellitus, schizophrenia, Alzheimer's disease, neurodegenerative disorders, hyperphosphatemia in dialysis patients, and as a radiosensitizer in cancer. Because nicotinamide is a safe and inexpensive dietary nutritional supplement that does not require FDA approval, two early phase clinical trials are ongoing at the University of Kansas Medical Center (KUMC) to test the effect of niacinamide in humans with ADPKD (Clinical Trials.gov. Identifier: NCT02140814). Once it can be proven to be safe and effective in PKD patients, it could be used to treat patients immediately.

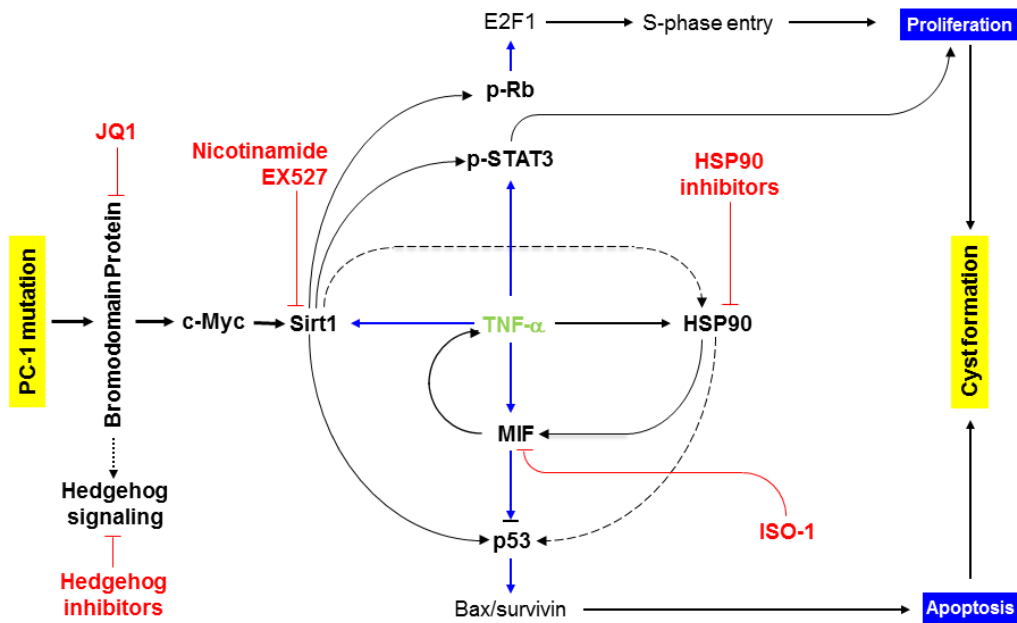


Figure 2. The relationship between epigenetic regulators and PKD-associated signaling. In this schematic diagram, we depicted the interplays of epigenetic regulators, Sirt1 and Bromodomain protein, with several already known PKD-associated signaling pathways, including tumor necrosis factor- α (TNF- α signaling, c-Myc signaling, STAT3 signaling, heat shock protein 90 (HSP90) signaling, macrophage migration inhibitory factor (MIF) signaling and Hedgehog signaling. The inhibitors of these pathways are marked in red. Solid lines indicate the already known connections among these signaling pathways in cystic renal epithelial cells, whereas the dashed lines indicate the potential connections of these signaling pathways in cystic renal epithelial cells based on the studies in other cell types.

SIRT2 and HDAC6 regulate ciliogenesis and SIRT2 contributes to abnormal centrosome amplification caused by loss of polycystin-1

The localization of PC1 and PC2 to the primary cilia has led to development of the “primary cilia” hypothesis for PKD, in that the abnormalities in primary cilia structure and function in tubular epithelia contribute to cyst initiation and development. The primary cilium is a microtubule-based organelle that originates from the one of the two basal bodies (centrioles) that form the core of the centrosome in quiescent cells. For cell division, the primary cilium has to be disassembled to liberate one of the captive centrioles of the centrosome which directs assembly of the bipolar spindle during mitosis (93, 94). Thus, cilia may passively affect the cell cycle. It has been demonstrated that HDAC6 and SIRT2, another member of class III HDACs, regulate the stability of microtubules through deacetylation of α -tubulin and regulate disassembly of cilia during the normal cell cycle (Figure 1) (26, 95). The fact that SIRT2 and HDAC6 are able to form a complex and α -tubulin binds to the SIRT2-HDAC6 complex *in vitro* (96, 97) suggest that SIRT2 and HDAC6 may regulate α -tubulin deacetylation and cilia size together. However, inhibition of either SIRT2 or HDAC6 alone is sufficient to induce hyperacetylation of α -tubulin and block cilia disassembly (26, 96) suggesting that SIRT2 and HDAC6 can regulate ciliogenesis independently. These results may explain why knockout of HDAC6 in mice does not cause hyperstable microtubules or persistent cilia (26) since SIRT2 may compensate for the loss of HDAC6 in knockout cells and organs. SIRT2-mediated α -tubulin deacetylation is able to regulate chromosomal segregation during mitosis to ensure normal cell division through affecting mitotic structures including the centrosome, mitotic spindle and midbody (98, 99). SIRT2 was upregulated in *Pkd1* knockdown mouse inner medullary collecting duct (IMCD3) cells and *Pkd1* knockout mouse kidney cells. This was responsible for the aberrant centrosome amplification and polyploidy induced by loss of PC1 (26). However, the role of SIRT2 in renal cyst development remains to be determined.

A BET bromodomain protein, Brd4, in ADPKD

Bromodomain proteins specifically bind to acetylated lysine residues on histone tails through bromodomains to regulate gene expression (55). Recently, we reported that a BET bromodomain (BRD) protein, Brd4, is a novel epigenetic regulator of ADPKD and a novel client protein of HSP90 (100). Brd4 was upregulated in *Pkd1* mutant mouse renal epithelial cells and tissues, which might be partially mediated by the chaperone activity of HSP90, leading the cells to escape proteasomal degradation. Targeting Brd4 with JQ1, a selective small-molecular inhibitor of BET bromodomain protein(s) (100), slowed cyst growth and kidney enlargement in two early stage *Pkd1* mutant mouse strains. Brd4 regulates the expression of c-Myc and p21, which further affects the phosphorylation of Rb and Rb mediated S-phase entry to regulate cystic renal epithelial cell proliferation (100). Our study

not only addresses how c-Myc is upregulated in PKD but also provides a rationale for targeting Brd4 with JQ1 as a potential epigenetic therapy in ADPKD. In addition, the association of Brd4 and HSP90 in ADPKD may also be a general mechanism for the upregulation of Brd4 in cancer cells.

Other potential functions of epigenetics in PKD

Epigenetic mechanisms in renal inflammation

Interstitial inflammation has been consistently reported in human and animal models of PKD. Whether renal inflammation is one of the primary factors for cyst initiation or a consequence of renal cyst formation is uncertain. However, its role in promoting cyst growth is supported by the findings that depletion of macrophages in kidneys of *Pkd1* conditional knockout mice and *cpk* mice, which develop renal cysts via the disruption of cystin (a cilia-associated protein), caused a significantly lower cystic index, reduced proliferation of cyst-lining cells, and improved renal function (7, 101). Inflammation in cystic kidneys is characterized by increased release of proinflammatory cytokines/chemokines, such as TNF- α , interleukins (ILs), and monocyte chemoattractant protein-1 (MCP-1), by tubular and endothelial cells, as well as dendritic cells and infiltrating leukocytes/monocytes (7, 20, 102). In recent years, epigenetic mechanisms have been shown to have a role in renal inflammation in kidney disease (102). Changes in histone acetylation and methylation were observed at inflammatory genes, including TNF- α and Ccl2/MCP-1 in various models of acute kidney injury (AKI) (103, 104). It has been found that endoplasmic reticulum stress can increase the levels of SET7, a histone methyltransferase, leading to increased histone 3 lysine 4 (H3K4) methylation at the Ccl2/MCP-1 promoter and its upregulation in the kidneys from diabetic db/db mice (105). Changes in histone 3 lysine 9 (H3K9) acetylation at inflammatory gene promoters had also been observed in diabetic mice models (106-108). In addition, the deacetylase Sirt2 also played a proinflammatory role in lipopolysaccharide-induced acute kidney injury by induction of NF- κ B activation and chemokine production in proximal tubular epithelial cells. Deletion of Sirt2 in mice delayed renal function decline and was protective against LPS induced infiltration of neutrophils and macrophages, and acute tubular injury (109). Together, these studies suggest that epigenetic mechanism may be involved in renal inflammation in PKD.

Epigenetic mechanisms in renal fibrosis

Severe interstitial fibrosis has also been associated with sustained enlargement of fluid-filled cysts in PKD. However, the mechanism for the development of interstitial fibrosis in

PKD remains elusive. It has been found that treatment of aberrant histone acetylation in experimental kidney fibrosis with TSA attenuates intra-renal inflammation and tubulointerstitial fibrosis in mice (110, 111). Administration of MS-275, a selective Class I HDAC inhibitor, and tubacin, a specific HDAC6 inhibitor, ameliorated fibrosis through inhibition of transforming growth factor (TGF)- β signaling, which is up-regulated in tissue fibrosis of several organs and causes fibroblast activation (112). Similarly, administration of vorinostat, the first FDA-approved HDAC inhibitor for clinical application, ameliorated diabetes-associated renal fibrosis in an animal model through the normalization of EGFR-mediated signaling (113). Other Class I and II HDAC inhibitors like phenylbutyrate and valproic acid could also be of benefit to experimental renal fibrosis (114-116).

In addition, it has been shown that DNMT1 is induced in experimental renal fibrosis (112) and Dnmt1 heterozygous knockout mice show ameliorated aberrant promoter methylation and reduction of renal tubulointerstitial fibrogenesis (112). RASAL1, a negative regulator of Ras signaling, is transcriptionally repressed due to its hypermethylation in experimental kidney injury, acute renal damage and chronic progressive fibrosis (112). Exposure to TGF- β further inhibited the expression of Rasal1 by promoting DNA methylation at its promoter via DNMT1, leading to Ras activation and increased fibrosis in fibroblasts, which can even be persistent after TGF- β is removed (112). Since only DNMT1 but no other member of the DNMT family is altered in kidney fibrosis, it suggests a predominant role of DNMT1 mediated DNA methylation in context of chronic progressive kidney disease. Together, the roles of HDACs and DNMT1 in PKD-associated interstitial fibrosis need be investigated.

Epigenetic mechanisms in hypertension

Mutations of PKD1 and PKD2 result not only in renal, hepatic and pancreatic cyst formation but also in cardiovascular complications characterized by an increased incidence of cardiac valve abnormalities and left ventricular hypertrophy (117-121). It has been suggested that ADPKD associated cardiovascular complications result from renal cyst growth induced cardiovascular hypertension, which occurs in patients at an earlier age than that in the general population even before any substantial reduction in renal function, and is associated with a rapid progression toward renal failure (122-125). It has been found that the mineralocorticoid aldosterone via upregulation of the tubular epithelial sodium channel (ENaC) regulates the disorders of Na⁺ transport, reabsorption, and excretion in the renal collecting duct, leading to abnormal blood pressure in humans (126). Under basal conditions, the transcription of ENaC subunit alpha (ENaC α) can be repressed by Dot1a, a lysine methyltransferase, mediated the methylation of histone 3 lysine 79 (H3K79) on the ENaC α promoter, keeping it constrained but poised for activation by aldosterone and other stimuli. Hypertension induced by high-salt diet is also associated with epigenetic mechanism

mediated upregulation of angiotensin I converting enzyme (ACE1), which has an important role in hypertension by activating the renin-angiotensin system, via increases in activation marks (H3KAc and H3K4me) and decreases in repressive mark (H3K9me2) at the promoter of ACE1 (127). Study of epigenetics in ADPKD-associated hypertension and its potential heritability is clearly warranted. Furthermore, the fact that cardiac hypertrophy also occurs in young ADPKD patients with normal blood pressure and renal function (128, 129) suggests that cardiac dysfunction in ADPKD patients does not develop solely in response to hypertension and/or renal failure. Additional epigenetic or environmental factors may be required and more investigations are anticipated.

Epigenetic mechanisms in regulating ADPKD associated signaling pathways

In addition to the well-documented PKD gene mutations that have been associated with cyst development, considerable attention is being focused on the participation of epigenetic events on the regulation of transcriptional and/or translational activities of PKD-associated signaling pathways, including HSP90 (11), STAT3/STAT6 (12-14), AMPK (15), Wnt/ β -catenin (16), GSK3 β (130), ILK/mTOR (8, 17), hedgehog (18), MIF (7) and NF- κ B/inflammation signaling (19, 20). Knockout of *Thm1* in mice resulted in renal cyst formation and the upregulation of the components of hedgehog signaling, including Gli1 and Gli2 (131). Knockout of *Pkd1* also induced the abnormal upregulation of Gli1 and Gli2 (131), which suggested that abnormal regulation of hedgehog signaling contributes to cyst development. A recent study found that the expression of Gli1 and Gli2 could be regulated by the bromodomain protein, Brd4, an epigenetic regulator that binds to acetylated histone tails, in NIH3T3 and mouse embryonic fibroblast (MEF) cells (132). Brd4 may regulate the expression of Gli1 and Gli2 through binding to the promoters of these genes in *Thm1* and *Pkd1* mutant renal epithelial cells. Additionally, the HSP90, MIF, GSK3 β and Wnt- β -catenin signaling pathways have been associated with epigenetic regulators (83, 133). It is highly possible that epigenetic event is involved in regulation gene expression and protein function of most, if not all, of PKD associated signaling pathways. Thus, determining the nature of epigenetic modifications and extent to which they occur on PKD-associated genes, and establishing how epigenetic events intertwine with PKD associated signaling pathways is highly significant for our understanding of the pathogenesis of PKD and can be achieved with the advance of epigenetic tools.

Tools to study epigenetic mechanisms in ADPKD

The importance of epigenetic alterations in ADPKD and in regulating ADPKD-associated signaling pathways is increasingly being appreciated. Epigenetics research has been spurred

by the technological breakthroughs in next generation sequencing (NGS) and advances in epigenomics platforms and data analysis tools that have aided in detecting epigenetic modifications such as histone modifications and DNA methylation, chromatin structure (open or condensed), as well as long-range interaction of enhancers in transcription regulation. Utility of these approaches to detect epigenetic changes at PKD genes and at PKD associated candidate genes should provide us an opportunity to gain new insights into the pathologies of PKD and uncover targets for novel epigenetic therapies.

The fact that *PKD1* is hypermethylated in gene-body regions, and its expression is downregulated in ADPKD (68) suggests that other ADPKD or ARPKD genes and genes downstream of PKD mutations may also be methylated. This can be determined by DNA methylation analysis. This mode of analysis, including Methylation Specific PCR, Bisulfite Sequencing, Bisulfite Pyrosequencing, and Genome Wide Methylation Analysis, is based on the treatment of genomic DNA with sodium bisulfite. Sodium bisulfite only deaminates cytosine but not 5-methylcytosine into uracil, which can be identified by sequencing to determine the DNA methylation status (134). During PCR and sequencing, uracil hydrogen bonds to adenine which will then hydrogen bond to thymine. Therefore, the unmethylated cytosines will become thymines and methylated cytosines will remain cytosines in the amplified sequence. DNA methylation analysis with genome-wide quantification of sodium bisulfite conversion-based cytosine method can be performed by NGS or the widely used Infinium Human Methylation 450K Bead-chips Assay (San Diego, CA), which is especially for large-scale clinical projects. In comparing with affinity-based methods, including methylated DNA immunoprecipitation-sequencing (MeDIP-seq) or methyl-CpG binding domain (MBD) protein-enriched genome sequencing (MBD-seq), Bisulfite Sequencing (bisulfite-seq) provides better resolution and genome wide coverage but it is more expensive and it involves more complex bioinformatics analysis.

Whole transcriptome profiling by NGS (for coding and noncoding genes) and epigenome-wide association studies, such as chromatin immunoprecipitation (ChIP) and ChIP-sequencing (ChIP-seq) analyses which combine immunoisolation of epigenetic marks and NGS, have been developed. These new tools can yield information on genome-scale dynamic changes and will help to identify novel epigenetic regulators and transcription factors involved in the expression of PKD genes or genes associated with PKD as well as novel downstream targets. A major advantage of NGS-based studies such as RNA-seq, ChIP-seq, bisulfite-seq, and others is that these unbiased approaches provide genome-wide and quantitative information unlike microarrays. However, before performing these studies, the more expensive costs and the complicated data analyses need to be considered. We believe that with advance in new and cheap technologies, epigenome association studies should be performed more frequently in experimental and clinical studies in PKD.

Perspectives and conclusions

Increasing evidence suggests a critical role for epigenetic modifications, including DNA methylation and histone/lysine deacetylation in ADPKD (21-26) (68). Studies have explored the potential beneficial effects of HDAC inhibitors in animal models of ADPKD. However, as the specificity and the mechanism of action of these inhibitors are not fully clear, more work, except for nicotinamide (vitamin B3), is needed before these inhibitors can be evaluated in humans. In order to screen novel HDAC inhibitors that could delay cyst growth in PKD mouse models, a zebrafish model was recently used and generated very promising results (71). Due to other epigenetic regulators, including HMTs and DNMTs, being also potentially involved in regulating cystogenesis, this novel screening approach may help to discover additional epigenetic modulators specific to PKD. In addition, we may test the epigenetic drugs that target histone-modifying enzymes in cancer treatment (135, 136) for preclinical treatment in PKD animal models. The links between epigenetic mechanisms and PKD associated signaling pathways have encouraged investigators to think about dual therapies, such as combining HDAC inhibitors with metformin or mTOR inhibitor in PKD treatment. It may be advantageous to sensitize cells by epigenetic therapy followed by treatment with chemotherapy, which targets PKD associated signaling pathways, including HSP90 (11), STAT3/STAT6 (12-14), AMPK (15), Wnt/ β -catenin (16), GSK3 β (130), ILK/mTOR (8, 17), hedgehog (18), NF- κ B/inflammation signaling (19, 20). In summary, epigenetics is clearly an exciting emerging field in basic and clinical studies of ADPKD, and the development of 'epigenetic therapies', specifically HDACs, have shown promising effects for PKD treatment. To investigate epigenetic mechanisms underlying cystogenesis is an exciting challenge but it may lead to a better understanding of cyst development and direct new therapeutic strategies of ADPKD. However, several roadblocks and challenges should also be overcome, including low specificity/selectivity of inhibitors of epigenetic regulators and unwanted side effects.

Conflict of interest

The author declares that he has no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 13

MicroRNAs and Polycystic Kidney Disease

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Abstract

MicroRNAs (miRNAs) are a class of small non protein-coding RNAs that function as inhibitors of post-transcriptional gene expression in plants and animals. Over a thousand different miRNAs are known to be encoded by the human genome, the majority of which are conserved in other species. miRNAs are essential for virtually all aspects of mammalian biology, including development of key organs such the brain, the heart, and the kidney. More importantly, miRNAs are implicated in the pathogenesis of numerous common human diseases, and pharmaceutical manipulation of miRNA function has emerged as an exciting new therapeutic approach for cancer and kidney diseases. Several lines of evidence have connected miRNAs to the pathogenesis of polycystic kidney disease (PKD). miRNAs

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are aberrantly expressed in cystic kidneys and this aberrant expression is thought to regulate key aspects of cyst pathogenesis such as cyst epithelial cell proliferation and apoptosis as well as dosage of the various cystic kidney disease genes. In this chapter, we briefly discuss the basic biology of miRNAs and their role in kidney development, and highlight the role of three miRNA families – miR-17 and related miRNAs, miR-200 family and miR-21- in the pathogenesis of PKD.

Key words: MicroRNAs; miR-17~92; miR-21; miR-200; Polycystic kidney disease

Introduction

MicroRNAs (miRNAs) are a class of small (approximately 22-nucleotide long) non protein-coding RNAs that function as inhibitors of post-transcriptional gene expression in plants and animals (1, 2). Drs. Victor Ambros, Gary Ruvkin and their colleagues first discovered miRNAs in the nematode *Caenorhabditis elegans* in the early 1990's (3, 4). For nearly a decade after this discovery, it was thought that miRNAs represented a phenomenon that was unique and limited to lower organisms. However, this assumption changed in the year 2000, when the first mammalian miRNA, called let-7, was described (5, 6). The discovery of let-7 sparked great interest in identifying new miRNAs, understanding miRNA biology in mammalian development, and studying the role of miRNAs in pathogenesis of common human diseases. Nearly fifteen years later, we now know that thousands of evolutionarily-conserved miRNAs are encoded by the human genome and that miRNAs are implicated in virtually all aspects of mammalian biology – ranging from embryogenesis and aging to metabolism and immunity. More importantly, miRNAs have emerged as key players in the pathogenesis of numerous human diseases (7, 8) such as cancer (9-14), diabetes (15), obesity (16, 17), infectious diseases (18) and even genetic disorders such as polycystic kidney disease (PKD) (19-24). A novel class of drugs, called antimirs and miRNA-mimics, that can manipulate miRNA function are currently in various stages of pre-clinical and clinical testing, raising hope that someday a miRNA-based therapeutic approach can be used to treat common human diseases (11, 25, 26).

MiRNAs: biogenesis, function and role in kidney development

Based on their genomic location, miRNAs can be classified into two groups: intragenic miRNAs or intergenic miRNAs. Intragenic miRNAs are located within introns or rarely exons of known protein-coding genes, and are generally co-transcribed with their host gene. In contrast, the intergenic miRNAs are located outside of any known protein-coding

genes and function as independent transcriptional units. Biogenesis of miRNAs involves RNA-polymerase II-dependent transcription of a relatively large capped and polyadenylated transcript known as primary miRNA (pri-miRNA). Pri-miRNA is processed by the RNase III endonuclease, Drosha, and its cofactor, Dgcr8 into smaller stem-looped structures known as precursor miRNAs (pre-miRNA). Pre-miRNAs are transported out of the nucleus by Exportin 5 into the cytosol, where further processing by a second RNase III enzyme, Dicer, leads to the generation of 19-25 nucleotide mature miRNA. The nucleotide sequence 2 through 8 at the 5'-end of the mature miRNA is referred to as the 'seed-sequence'. The mature miRNA associates with the miRNA-induced silencing complex (miRISC), where Watson-Crick base-pairing between the seed-sequence of a mature miRNA and complementary sequences primarily located within 3'-UTRs of mRNAs results in post-transcriptional gene silencing (Figure 1). In this manner, miRNAs function as sequence-specific inhibitors of mRNA translation(27). miRNA-mediated regulation of mRNA expression is likely to be extremely complex. Bioinformatic algorithms predict that each miRNA could potentially inhibit thousands of mRNAs(28-35). Each mRNA, in turn, may possess binding sites for numerous unique miRNAs. Additional factors that further complicate the regulation of mRNA expression by miRNAs include the secondary structure of the mRNA and binding of proteins to mRNAs in close proximity to miRNA-binding sites. Recent studies have shown that some miRNAs can be produced independent of the canonical Drosha-Dicer pathway and that miRNAs can inhibit mRNA translation by binding to coding regions and 5'-UTRs of mRNAs. Thus, a lot remains to be learned about the basic miRNA biology.

miRNAs are implicated in development of various organs, including the kidney (36, 37). Kidney development involves interactions between two embryonic structures, the metanephric mesenchyme (MM) and the ureteric bud (UB). The MM is a precursor tissue composed of renal progenitor cells that gives rise to glomeruli and nephrons. The UB is a 'T'-shaped epithelial structure that eventually gives rise to the collecting ducts. Signals from the UB induce the progenitor cells of the MM to undergo differentiation. Conversely, the MM sends signals to the UB, which causes UB to undergo branching. This process of reciprocal signaling between the MM and UB is repeated innumerable times to eventually give rise to nearly one million nephrons and collecting ducts. Mutations of cystic kidney disease genes, particularly those implicated in childhood forms of PKD are known to disrupt normal kidney development. Therefore, pathogenesis of some forms of PKD can be traced back to abnormal kidney development. miRNAs have been shown to regulate virtually all processes in kidney development (36-39). Inhibiting miRNA function in the UB or MM prevents UB branching and MM differentiation, respectively, ultimately resulting in renal agenesis or dysplastic kidneys. Inhibiting miRNA function at later stages of kidney development, specifically in the

elongating renal tubules without affecting UB branching and MM differentiation, results in the formation of numerous tubular and glomerular cysts, a phenotype that is reminiscent of PKD (40). Intriguingly, the proposed mechanism by which miRNAs may regulate normal renal tubule elongation is by modulating the expression of various cystic kidney disease genes, in particular the autosomal dominant polycystic kidney disease (ADPKD) gene *PKD1*(40). Thus, these observations provide the earliest and a direct link between miRNAs, kidney development and cyst pathogenesis.

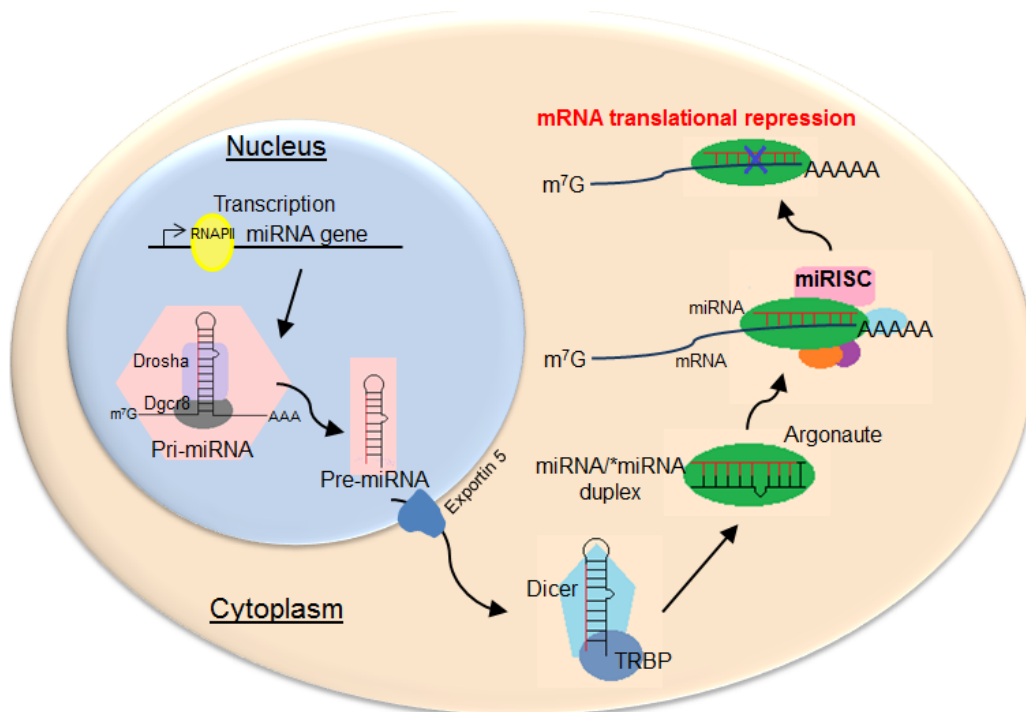


Figure 1. A schematic of miRNA biogenesis and function in animals. miRNA biogenesis begins in the nucleus, where RNA-polymerase II-dependent (RNAPII) transcription of a relatively large capped and polyadenylated transcript known as primary miRNA (pri-miRNA). Pri-miRNA is processed by the RNase III endonuclease, Drosha, and its cofactor, Dgcr8 into smaller stem-looped structures known as precursor miRNAs (pre-miRNA). Pre-miRNAs are transported out of the nucleus by Exportin 5 into the cytosol, where further processing by a second RNase III enzyme, Dicer, leads to the generation of mature miRNA. The mature miRNA associates with the miRNA-induced silencing complex (miRISC), where Watson-Crick base-pairing between the seed-sequence of a mature miRNA and complementary sequences primarily located within 3'-UTRs of mRNAs results in post-transcriptional gene silencing.

MiRNAs that regulate PKD pathogenesis

Emerging evidence from studies performed on rodent models and bio-specimens obtained from human ADPKD patients suggests that aberrant expression of many miRNAs may underlie disease progression in PKD. In this section, we will primarily discuss the role of three families of miRNAs in the pathogenesis of PKD; miR-17 and related clusters, miR-200 and miR-21.

miR-17 and related miRNAs

The miR-17 family of miRNAs consists of fifteen miRNAs that are located as three distinct clusters on different chromosomes. In humans, the first cluster - miR-17~92, is located within the third intron of the non-protein coding gene, *MIR17HG* (*C13orf25*) on chromosome 13 (13q31.1-q33-1). The cluster consists of miR-17, miR-18a, miR-19a, miR-20a, miR-19b-1, and miR-92-1. The second cluster - miR-106b~25, is located in the 13th intron of *MCM7* on chromosome 7 (7q22.1) and consists of miR-106b, miR-93, and miR-25. The third cluster - miR-106a/363 is located on chromosome X (Xq26.2) and consists of miR-106a, miR-18b, miR-20b, miR-19b-2, miR-92a-2 and miR-363. Based on their seed sequences, the fifteen miRNAs can be grouped into four families - the miR-17, the miR-18, the miR-19, and the miR-92 family. The four families target different mRNAs; however they are predicted to repress multiple mRNA targets within the same pathways, thus regulating entire signaling nodes.

The miR-17~92 cluster is expressed at high levels in embryonic cells and is essential for normal development of various organs (41, 42). Microdeletions of the miR-17~92 cluster cause Feingold syndrome, a human developmental disorder that is characterized by defects in the skeletal and gastrointestinal system (43). Some patients with Feingold syndrome also have mental retardation and kidney and heart developmental abnormalities. Consistent with these findings, deletion of miR-17~92 in mice produces skeletal, heart, brain and kidney developmental defects (44, 45). Several lines of evidence have proved that the miR-17~92 cluster is a bonafide oncogenic miRNA cluster (10, 46). First, the miR-17 family and related miRNAs are upregulated in various human cancers (47), which include those of the kidney, colon, breast, prostate, stomach, and the pancreas. The oncogenic transcription factor, c-Myc binds to the promoter region of miR-17~92 and activates its transcription (48, 49). Second, forced expression of these miRNAs aggravates, whereas inhibiting miR-17~92 slows, cancer growth in mice (50-53). Lastly, the miR-17~92 cluster promotes proliferation of cells through direct and indirect inhibition of numerous tumor suppressor genes and promotes proliferation of cells.

While the miR-17~92 cluster and related miRNAs have been studied extensively in cancer, their role in kidney diseases is not completely understood. Studies by our group have conclusively proved that the miR-17~92 cluster is pathogenic in PKD (23). The expression of the miR-17~92 cluster is increased in orthologous as well as non-orthologous mouse models of PKD. Kidney-specific over-expression of the miR-17~92 cluster leads to cyst formation. Conversely, deletion of the cluster in a mouse model of PKD ameliorates the cystic phenotype, improves renal function and prolongs survival. One of the mechanisms through which the miR-17~92 cluster aggravates to cyst growth is by promoting proliferation of the cyst epithelial cells. Overexpression of the miR-17~92 cluster in the kidney leads to increased proliferation of cells lining the cysts, while deletion of the cluster in a model of PKD, decreased proliferation. A novel mechanism for cyst growth in PKD has been uncovered, which involves post-transcriptional regulation of cystic kidney disease genes by members of the miR-17~92 cluster. The ADPKD genes, *Pkd1* and *Pkd2* harbor conserved binding sites within their 3'-UTRs for members of the miR-17 family, while the hepatocyte nuclear factor 1-beta gene (*HNF-1 β*) has conserved binding sites for the miR-25 family within its 3'-UTR. HNF-1 β is an epithelial-specific transcription that regulates the expression of multiple cystic kidney disease genes (54, 55). In humans, mutations of *HNF-1 β* produces cystic kidney disease and early-onset diabetes mellitus, a syndrome called renal cysts and diabetes (RCAD) (56). Several lines of evidence indicate that miR-17 represses these cystic genes *in vitro* and *in vivo*. In mouse kidneys, over-expression of the miR-17~92 cluster leads to decreases in the expression of *Pkd1*, *Pkd2*, *Pkhd1* and *Hnf-1 β* , while kidney-specific deletion of the miR-17~92 cluster in a PKD mouse model leads to upregulation of the same set of cystic genes. In cultured renal epithelial cells, reporter assays indicate that miR-17 represses *Pkd1* and *Pkd2*, while miR-25 represses *Hnf-1 β* by directly binding to their 3'-UTRs. In addition, mutation of the miR-17 and miR-25 binding sites within the 3'-UTRs of *Pkd1*, *Pkd2* and *Hnf-1 β* , respectively, abrogated the miRNA mediated repression. The autosomal recessive polycystic kidney disease (ARPKD) gene, *Pkhd1* does not harbor binding sites for miR-17; however, its expression is directly regulated by *Hnf-1 β* , which explains the change in its levels in miR-17 overexpressing cells and miR-17~92 knockout kidneys (57). In addition, bioinformatic analysis indicates that a number of genes that are mutated in humans with cystic diseases and developmental disorders are targets of the miR-17/18/19 and 25 families. Thus, miR-17 may promote cyst growth in PKD by directly and indirectly modulating the gene dosage of a large network of cystic kidney disease genes. Reduced gene dosage of ADPKD genes has been proposed as a new mechanism for cyst pathogenesis (58-60). The hypothesis states that kidney cysts form in ADPKD patients because the dosage of ADPKD genes falls below a critical threshold. Mutations that moderately reduce ADPKD gene dosage cause a milder form of the disease, whereas more deleterious mutations that severely reduce ADPKD gene dosage cause an aggressive form of disease. In this scenario, miR-17 may act as a modifier of disease

progression in ADPKD. Increased levels of miR-17 can further reduce ADPKD gene dosage and aggravate disease progression. Importantly, inhibiting miR-17 may increase the ADPKD gene dosage and retard disease progression.

miR-200 miRNA family

The miR-200 family comprises five members – miR-200a, miR-200b, miR-200c, miR-141, and miR-429. These miRNAs are located as two clusters on separate chromosomes. In humans, the miR-200b~miR-429 cluster is located on chromosome 1(1p36.33), while the miR-200c and miR-141 cluster is located on chromosome 12 (12p13.31). Based on their seed sequences the five miRNAs are divided into two subgroups – group I comprises miR-200a and miR-141 while group II comprises miR-200b, miR-200c and miR-429; however, these two groups regulate many of the same mRNA targets. Several lines of emerging evidence suggest that the miR-200 miRNA family plays an important role in renal tubule development and cyst pathogenesis. First, the expression of miR-200 family members is highly enriched in the normal kidney tubules whereas its expression is reduced in injured kidney tubules. Second, kidney tubule-specific knockout of the miRNA biogenesis enzyme, *Dicer*, leads to significant down regulation in the expression of all five members of the miR-200 family and formation of kidney tubule-derived cysts (40). Furthermore, miR-200 knockdown in cultured renal epithelial cells inhibits tubulogenesis and produces cyst-like structures, thus implicating miR-200 in the maintenance of normal renal tubule structure and preventing cyst formation. Third, miR-200 is known to regulate the expression of the ADPKD gene, *PKD1*. Bioinformatic analysis of *PKD1* 3'-UTR has identified two evolutionary-conserved binding sites for the miR-200 members. The miR-200 family members directly bind to *PKD1* 3'-UTR and inhibit its translation. Thus, miR-200 may regulate cyst pathogenesis through modulation of *PKD1* gene dosage. Fourth, the transcription of miR-200 is regulated by another cystic kidney disease gene, *Hnf-1 β* (61). In mice, kidney tubule-specific deletion of *Hnf-1 β* results in decreased expression of miR-200 miRNA family members and causes renal cysts. HNF-1 β is known to promote the expression of key cystic kidney disease genes including the ADPKD gene *PKD2* and the ARPKD gene *PKHD1*. Interestingly, HNF-1 β binds to a promoter region upstream of the miR-200 gene and directly controls the transcription of the miR-200b~429 cluster via a long non-coding RNA. Thus, along with *PKD2* and *PKHD1*, miR-200 belongs to a network of cystic kidney disease genes regulated by HNF-1 β .

The cellular mechanism by which miR-200 regulates cyst pathogenesis may involve epithelial-to-mesenchymal transformation (EMT), a process in which epithelial cells lose polarity (e.g. apical-basal polarity) and acquire mesenchymal properties such as increased migratory capacity. miR-200 is known to maintain epithelial integrity and inhibit EMT, at

least in part, through direct inhibition of mesenchymal transcription factors *ZEB1*, *ZEB2* and transforming growth factor- β (*TGF- β 2*), a potent inducer of EMT (62-68). However, renal tubule epithelia in kidneys of *Dicer* and *Hnf-1 β* mutant mice do not appear to undergo EMT (40). These cells might be undergoing 'partial EMT' wherein they simultaneously express both epithelial and mesenchymal markers. This has been observed in the kidney-specific HNF-1 β knockout mice, as the expression of miR-200 targets - *Zeb2* and *TGF β 2* are increased several fold, while the expression of epithelial polarity protein, E-cadherin, is unchanged (61). While the role of partial EMT in aggravating cyst growth currently remains uncharacterized, partial EMT of renal tubule epithelia has been recently shown to promote renal tubule injury and kidney fibrosis (69). In summary, miR-200 members help maintain renal tubule homeostasis by preventing cells from undergoing partial EMT and regulating the dose of genes involved in cystic kidney disease.

miR-21

In humans, miR-21 is located on chromosome 17q23.2, where it overlaps with a protein-coding gene called Vacuole membrane protein 1 or *Vmp1*. Despite its intergenic location, the transcription of miR-21 is regulated independently of its host gene *Vmp1* through its own unique promoter. Even though miR-21 is expressed at high levels in multiple normal tissues, such as the heart, the liver and the kidney, miR-21 knockout mice display no overt phenotype, are fertile and live a normal life span (70). Thus, miR-21 is dispensable for normal development. Instead, the physiologic function of miR-21 may be in aiding organ regeneration after injury by promoting proliferation and/or inhibiting apoptosis of cells (71). This function of miR-21 is often 'hijacked' in the context of cancer to fuel the growth of malignant cells. miR-21 is dubbed oncomir because it is frequently amplified in multiple forms of cancers, where it is thought to promote proliferation and inhibit apoptosis of malignant cells by directly repressing a network of tumor suppressor genes (12, 72-74). Another disease in which miR-21 has been extensively studied is tissue fibrosis. While miR-21 may be necessary for recovery after acute injury, persistent elevation of miR-21 is thought to promote fibrosis (75, 76), particularly in the kidney. Several lines of converging evidence have shown that inhibiting miR-21 retards the progression of kidney fibrosis in murine models (77-79). These observations have provided the basis for initiating clinical trials to assess the safety and therapeutic efficacy of antimir-21 drugs in patients with Alport syndrome, a genetic condition that cause progressive kidney fibrosis.

Given that cancer and PKD share several common characteristics, it is not surprising that miR-21 has also been implicated in the pathogenesis of PKD. Our initial observations indicate that miR-21 is markedly up-regulated in multiple rodent models of PKD. miR-21 expression is also increased in cyst epithelial cells from human ADPKD samples. An

intriguing aspect of miR-21 transcriptional regulation is that its expression is activated by cAMP-CREB signaling. Thus, aberrant cAMP signaling may mediate its cyst promoting effects, at least in part, through up-regulation of miR-21. miR-21 expression is also activated by other cyst promoting pathways such as Janus Kinase (JAK)/STAT and mitogen activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathways indicating that multiple pathogenic signaling pathways converge and cooperatively activate miR-21 expression. Importantly, inhibiting miR-21 expression slows cyst growth in a mouse model of ADPKD. Like cancer, miR-21 aggravates cyst growth by inhibiting apoptosis and promoting the survival of cyst epithelia. A potential molecular mechanism by which miR-21 aggravates cyst growth may be through direct inhibition of the pro-apoptotic, tumor suppressor *Pdcd4*. Interestingly, *Pdcd4* knockout mice spontaneously develop kidney cysts indicating that *Pdcd4* inhibition is sufficient to produce cysts. In summary, increased levels of miR-21 may promote disease progression in ADPKD by promoting the survival of cyst epithelial cells.

Other miRNAs

Several other microRNAs have been implicated in the pathogenesis of PKD. Microarray-based screening approaches have been used to identify miRNAs that are aberrantly expressed in mouse and rat models of PKD as well as human ADPKD samples (80, 81). These studies have shown that miR-214, miR-185, miR-146b, miR-503, miR-34a and miR-10 are upregulated whereas miR-204 and miR-488 are downregulated in cystic kidneys compared to normal kidneys. An aberrant miRNA expression profile has also been observed in epithelial cells derived from bile duct cysts from animal models of ARPKD (82, 83). Though insightful, further studies will be required to determine if the differentially expressed miRNAs directly promote PKD pathogenesis.

The potential for a miRNA-based therapeutic approach in PKD

The basic understanding of miRNA biology and the fact that miRNAs appear to play direct pathogenic roles in various diseases has led to the development of novel miRNA-based therapeutic approaches. These approaches involve the use of synthetic oligonucleotides called antimirs and miRNA-mimics (25, 26, 84, 85). Antimirs harbor sequences that are complementary, whereas miRNA-mimics harbor sequences that are identical to the sequences of a mature miRNA of interest. Once inside the cell, the antimirs bind to the targeted miRNAs and inhibit their function. In contrast, the miRNA-mimics associate with the miRISC complex and 'mimic' the function of the targeted miRNAs. While both antimirs and miRNA-mimics are being developed as novel drugs, antimirs have shown more early

promise. Antimirs possess several characteristics that make them an ideal therapeutic agent for a chronic disease such as PKD. Antimirs inhibit miRNA function most efficiently in the kidney and the liver, the two organs most affected by ADPKD and ARPKD. Antimirs can be self-administered (similar to insulin) and appear to be safe with no adverse effects reported in human clinical trial (18). Interestingly, antimirs have a long duration of action (as long as 4 weeks) (26) and may need to be taken only once every few weeks. These attributes are particularly well-suited for treatment of a chronic disease like ADPKD, which will require life-long therapy. As highlighted in above sections, miR-17 and miR-21 directly promote cyst growth in PKD. Therefore, it is tempting to speculate that antimir-mediated inhibition of miR-17 and/or miR-21 can be used as a therapeutic approach to slow cyst growth. Another possibility is that antimirs may be used along with other drugs, such as tolvaptan(86), to synergistically slow disease progression in ADPKD.

Despite this early excitement, significant challenges remain with regards to using antimirs to treat ADPKD. While antimirs are easily delivered to normal kidneys, delivery to cystic kidneys may not be that straightforward because the cystic kidney is severely anatomically distorted. Moreover, the majority of cysts in PKD arise in the distal segments of nephron and collecting ducts, while the antimirs primarily appear to be taken up by proximal tubules. Finally, while antimirs are well-tolerated in short term clinical trials, whether they can be safely tolerated for long periods of time is not known. Recently, early-stage clinical trials have been launched to test the therapeutic potential of antimir-21 in a genetic disorder called Alport syndrome that causes kidney fibrosis and like ADPKD will also require life-long therapy. These studies will provide important insights into whether antimirs can be safely used for long-term therapy.

Conclusion

miRNAs have emerged as important new regulators of normal kidney development as well as being involved in the pathogenesis of many kidney diseases, including PKD. At least three different miRNAs families – miR-17 and related miRNAs, miR-200 family and miR-21- have been implicated in the pathogenesis of PKD (Figure 2). These miRNAs are thought to promote cyst pathogenesis through regulation of key aspects of cyst pathogenesis such as proliferation and apoptosis of cyst epithelia, and direct regulation of PKD gene dosage. New approaches involving antimirs that pharmaceutically inhibit miRNA function holds promise as a novel therapeutic strategy for PKD.

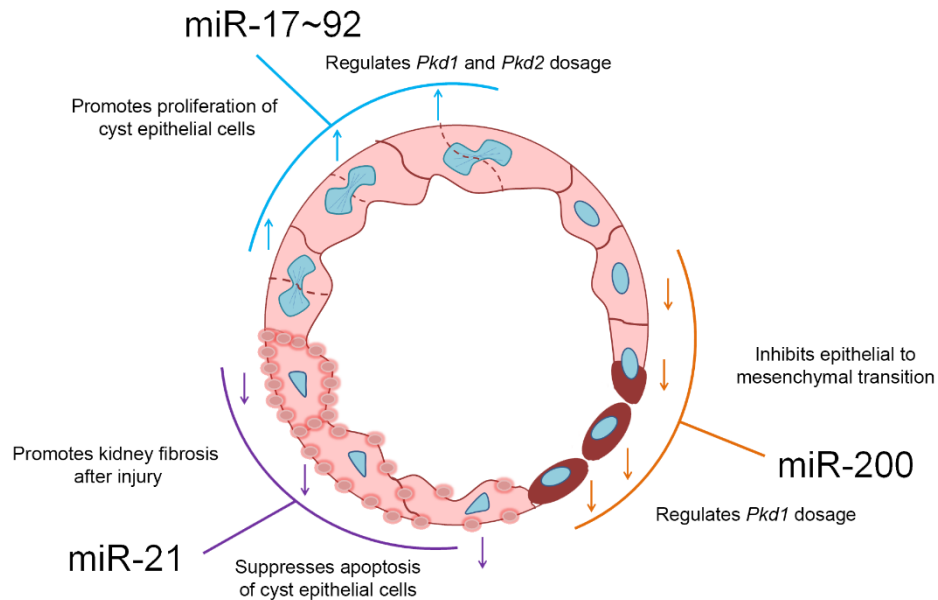


Figure 2. A model for potential mechanisms by which miRNAs regulate cyst growth. miR-17 promotes proliferation of cyst epithelia and reduces ADPKD gene dosage. miR-21 inhibits apoptosis and thus, promotes survival of cyst epithelial cells. miR-200 reduces *Pkd1* gene dosage and inhibits epithelial to mesenchymal transition (EMT). Loss of miR-200 may result in partial-EMT and increased *Pkd1* dosage, which collectively may aggravate cyst growth.

Conflict of interest

The authors declare that they have no conflict of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 14

Role of Inflammation in Polycystic Kidney Disease

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Abstract

Polycystic kidney disease (PKD) is a genetic disorder that is characterized by progressive growth of multiple cysts in the kidneys. Two forms of the disease exist, autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD). ADPKD is the most common form of genetic disorder of the kidney. In the United States about 600,000 people have PKD. This disease eventually leads to end stage kidney disease (ESKD) which may take as long as three decades to develop and requires renal replacement therapy. PKD is the fourth largest cause of kidney failure. Interstitial inflammation is a common component of chronic kidney disease (CKD) and, it is a better prognostic marker, than glomerular

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health, for progression of the disease or kidney function. PKD is no exception. Markers of inflammation are present much earlier than detectable cyst growth and correlate with the disease progression. While in other kidney diseases interstitial inflammation is mostly associated with interstitial fibrosis, in PKD inflammation is also linked to cyst progression. Inflammatory cells such as macrophages have been reported in both human and experimental animal models of PKD, with the degree of macrophage infiltrate associated with disease progression in humans. In animal models of PKD, macrophages have been associated with cyst growth and deteriorating renal function. What may differentiate PKD from the rest of the CKD is that experimental evidence suggests a direct role of PKD genes in regulating expression of some of the pro-inflammatory chemoattractants such as monocyte chemoattractant protein-1 (MCP-1) and other cytokines. Indeed, increased urinary levels of MCP-1 correlate with the progression of disease in humans. This chapter will highlight some of the work that provides evidence for the role of inflammation in disease progression in PKD.

Key Words: Chemokines; Cytokines; Inflammation; Macrophages

Introduction

Polycystic kidney disease (PKD) is one of the most common genetically inherited causes of chronic kidney disease (CKD) that leads to end stage kidney disease (ESKD) requiring renal replacement therapy (1). PKD may be inherited as either an autosomal dominant (ADPKD) or autosomal recessive trait (ARPKD). ADPKD is the most common form of PKD and is estimated to account for 7-10% of ESKD patients (1-4). ADPKD is caused by inherited or *de novo* mutations in *PKD1* and/or *PKD2* genes that encode ciliary proteins, polycystin 1 (PC1) and polycystin 2 (PC2) respectively (5, 6). The majority of cases are attributed to mutations in the *PKD1* gene (~ 85%) (7, 8). PKD exhibits tubular dilatation and bilateral fluid-filled cyst formation (4, 9, 10). Cysts in ADPKD develop slowly over a period of decades until ~ 60% of the parenchyma is destroyed after which renal function starts deteriorating rapidly, usually beyond the fifth decade of life (11) (Figure 1). It is estimated that cysts originate from not more than 1% of the total nephrons (12). Cysts form in cortex and medulla, although the medullary cysts may impact a larger number of nephrons. Since a single collecting duct drains ~ 2,800 tubules (13), it has been proposed that a cyst in a single collecting duct (in the papilla) could theoretically impact/impede 2,800 tubules (that may otherwise be normal) and negate their contribution to concentrating urine (14). Similarly, it has been further proposed that a cyst of ~ 400 μm in diameter could potentially block at least 32 adjacent tubules (explained in detail and illustrated in (14). In addition, these

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expanding cysts (like fluid-filled balls) compress the surrounding tubuli and microvasculature, impeding both urine and blood flow. This leads to focal intrarenal hypoxia and cascades into a chronic inflammatory response causing further damage that is independent of the effects of the underlying genetic mutations. This is one mechanism whereby a small number of affected tubuli (~ 1%) could cause such extensive and progressive damage.

During the last two decades enormous understanding has been gained about molecular and cellular mechanisms that lead to cyst formation and expansion (8). Experimental evidence implicates abnormal / dysfunctional proteins associated with PKD1/2, cyst expansion causing anatomic distortion of the kidneys, factors present within the cysts, dedifferentiation of tubular epithelial cells (before the cysts separate from the tubule) and a multitude of signaling cascades (1, 11). However, another component that may contribute to the progression of PKD is the chronic interstitial inflammation. This chapter will bring to the fore our current understanding of what role, if any, inflammation might play in overall progression of PKD.



Figure 1. Gross specimen showing massively enlarged kidney with multiple cysts. Picture courtesy of Dr. Moeckel, Department of Pathology, Yale School of Medicine, New Haven, CT - USA.

Brief clinical description

Bilateral kidney cysts that at later stages lead to impaired function characterize ADPKD, which occurs with equal frequency among males and females. The disease generally manifests after the third or fourth decade and is often diagnosed upon a sudden episode of hematuria, renal pain or accidental finding on radiological examination, or presentation with hypertension (11). Hypertension precedes any significant decline in glomerular filtration rate (GFR) and may already be present in the third decade (15). Thus, in a majority of patients renal dysfunction remains undiscovered until GFR declines to abnormal levels. Typically GFR may not decline in PKD until the total kidney volume has exceeded five times that of a normal kidney volume and the decline thereafter averages 4-7 ml.min⁻¹.yr⁻¹ (16, 17) (18). The disease may be accompanied by several extrarenal manifestations that include liver and pancreatic cysts, cardiac valve defect, pericardial effusion, brain aneurysm (~8% of patients), abdominal wall hernia and hypertension (16, 19-21).

Initiation of cyst formation

The disease progression in ADPKD may be thought to have two parts – one of cyst initiation and the second of progression or expansion accompanied by declining renal function. The fundamental pathophysiology stems from the loss of PC1 and/or PC2 function due to the mutations in their respective genes. PC1 and PC2 have been shown to function in a complex as a transient receptor potential channel regulating intracellular calcium homeostasis either at the cilia or endoplasmic reticulum (ER) (22-25). In the primary cilium, the PC1/PC2 complex is believed to act as a sensor for inducing the influx of extracellular calcium following flow-mediated shear stress (26-28). In the ER however, it functions as a calcium release channel (22, 23). Experimental evidence suggests that following the loss or dose reduction of PC1/PC2 proteins there is an increase in epidermal growth factor (EGF) and cAMP-dependent tubular epithelial cell proliferation, tubular cell apoptosis (in very early stages), abnormal fluid secretion, loss of apico-basal polarity and changes in the extracellular matrix (29-32). The role of cAMP signaling in ADPKD has been studied extensively and has led to approval of the first drug, Tolvaptan (a vasopressin receptor antagonist) in Canada and Europe, discussed in detail elsewhere (18, 33-35). It is awaiting approval in the United States. A recent study in a hypomorphic mouse model of PKD1 demonstrated that adding a somatostatin receptor inhibitor, Pasireotide, along with Tolvaptan, had an additive effect on reducing cyst burden as well as fibrosis (36). Both, Tolvaptan and Pasireotide target adenylyl cyclase 6, the predominant form of adenylyl cyclase in the collecting duct.

Although all cells of the body carry the PKD mutations, only a small number of tubules give rise to cysts (11). This phenomenon is attributed to occurrence of a second somatic hit in the normal copy of the gene which then leads to loss of function in a small subset of tubular epithelial cells and the cyst formation gets initiated (37-40). ESKD occurs at an earlier age (54 years) in patients with a PKD1 mutations than in those with PKD2 mutations (70 years) (41).

This is thought to be due to a greater number of cysts with PKD1 mutations as compared to PKD2 mutations. The rate of cyst growth is not thought to be different. A recent study has shed new light on the function of polycystins, cilia and how their interplay might regulate cyst formation. The study demonstrates that polycystin acts as suppressor while cilia provide a positive stimuli for cystogenesis suggesting that this could be yet another mechanism for cyst growth independent of ERK, cAMP or mammalian target of rapamycin (mTOR) pathways (42).

Regardless of the signaling mechanisms, after acquiring the second hit, monoclonal expansion of the affected cells initiates the cyst development. Persistent proliferation of these cells leads to radial expansion of the tubule eventually taking a shape of a sac-like bud (illustrated in (14)). It is interesting to note that normal tubular epithelial cells such as inner medullary collecting duct cells have a propensity to assemble into a lumen-containing tubule-like structure, when suspended in a 3D collagen/matrigel mixture (43). In contrast, tubular cells lacking PC1 or PC2 by default form cyst-like structures suggesting a phenotypic switch due to the lack of polycystins (44, 45). Cysts eventually separate from the tubule as independent 3D structures and create obstruction in the parenchyma by compressing adjacent tubules and vasculature thereby creating a secondary phenomenon resembling obstructive nephropathy. Indeed similarities have been drawn between ADPKD and the rodent unilateral ureteral obstruction (UUO) model of nephropathy (14). Both induce a similar array of chemokines and cytokines (14). However, in humans the cysts grow slowly at least for the first three or four decades. Despite slow cell proliferation, the kidney volume in ADPKD progressively increases at a rate of 5-6% annually suggesting that cell proliferation alone may not account for the expansion of the renal volume (16, 46). The phenotypic changes in the mutant cells paired with the distortion of the otherwise normal tubules and vessels, sets in motion an inflammatory response that has the hallmark of an acute reparative response but ends up being chronic and pathological leading to fibrosis. Indeed, very early on, researchers had noted significant changes in matrix and cell dedifferentiation in histopathology analysis of kidney tissue from ADPKD patients (47, 48). Inflammation in chronic kidney disease (CKD) has been known for over a decade now. Its potential contribution to the progression of ADPKD has been in focus only recently.

Inflammation in CKD

Inflammation is a protective physiological response that is initiated to neutralize the initial cause of cell or tissue injury (such as a pathogen) and to clean off the by-product of the injury, necrotic or apoptotic cells or tissues (49). However, an inflammatory response must be tightly regulated to avoid any damaging effects that could lead to mortality (49). Several studies have demonstrated the importance of inflammation in CKD (50-52) and have indicated that persistent inflammation also increases risk for cardiovascular disease (CVD)-dependent mortality in ESKD patients (53-55). CVD is also responsible for ~ 80% deaths in ADPKD (56, 57) highlighting a potential detrimental role of inflammation in ADPKD.

There are two main types of inflammation processes, active and chronic. Active or acute inflammation is triggered within hours/days. Histologically, it is defined by the presence of neutrophils that have migrated from the bone marrow to the site of injury. It also includes eosinophils and basophils (mast cells), the latter typically seen in response to an allergy. In contrast, chronic inflammation occurs within weeks/months. Histologically, it is represented by the presence of mononuclear cells – lymphocytes and macrophages (differentiating from circulating monocytes). However, active inflammation, tissue repair and destruction may co-exist with chronic inflammation, as is the case in CKD.

Inflammation in PKD

Subclinical inflammation has been linked with progression of CKDs (52, 58). Similar evidence in the progression of ADPKD is scarce. Generally speaking, PKD is not an inflammatory disorder. However, evidence is gradually accumulating to indicate onset of inflammation very early in the course of the disease. The presence of an inflammatory component in humans with PKD, as well as rodent models of the disease, has been reported in a few studies. For example, interleukin (IL)-1 β , tumor necrosis factor- α (TNF- α and IL-2 (proinflammatory cytokines) were observed in cyst fluid of human PKD kidneys (59-61). Monocyte chemoattractant protein-1 (MCP-1) was detected in urine of ADPKD patients and urinary MCP-1 levels correlated positively with the progression of ADPKD in humans (61). Similarly, increased *Mcp-1* expression was reported in Han:SPRD rats, a nonorthologous model of ADPKD (62). More recently, genes involved in the innate immune response were found to be the most significantly upregulated genes in severely cystic *cpk* mice, a nonorthologous mouse model of ARPKD (63). These findings were further validated by Zhou and co-workers who found significantly higher expression of the markers of monocytes and macrophages in severely cystic *cpk* mouse kidneys. Specifically, they found CD14, a marker of alternatively-activated macrophages, to be highly upregulated and activated (64). CD14 is a pattern recognition receptor that engages with Toll-like receptors (TLRs) to activate innate immune response. In yet another recent study, a systems biology approach revealed a rich profile of genes associated with inflammatory responses in cystic human tissues from five PKD1 mutant kidneys (65). Soon after, in a cross-sectional study, Menon and co-workers reported that hypertensive ADPKD patients with eGFR of 25 to 60 ml/min had higher levels of inflammatory markers such as C-reactive protein (CRP) and IL-6 compared to healthy controls, normotensive ADPKD with eGFR >60 and hypertensive ADPKD with eGFR of >60 (66). Although the study size was relatively small, the data nonetheless indicate the presence of inflammation early in ADPKD even when the renal function is preserved. Moreover, the authors also found a linear increase in these inflammatory markers with declining kidney function, suggesting a causal relationship of inflammation to the disease progression. In contrast, markers of oxidative stress, while high across ADPKD with varied renal function, did not change with the progression of disease. Kocyigit and co-workers examined a temporal relationship between ADPKD, hypertension and the loss of renal function in fifty patients with ADPKD who did not yet have hypertension (67).

The results indicated increased pulse wave velocity and arterial stiffness occurred even before detection of hypertension and reduced eGFR. The same group recently determined that pentraxin-3 (PTX-3) is a better marker of inflammation and endothelial dysfunction than CRP. In ADPKD patients, PTX-3 had already increased even before any changes in their blood pressure or change in eGFR. PTX-3 correlated positively with proteinuria and uric acid, once again suggesting that inflammation occurs much earlier and may contribute significantly to the progression of disease (67). However, these vascular abnormalities are signs of systemic subclinical inflammation (68) suggesting that inflammation (vascular) predates any measureable renal deterioration. Thus, gradually, evidence is accumulating that suggests a potentially significant role of inflammation in the progression of ADPKD.

How might inflammation contribute to disease progression in ADPKD?

When in 1970, it was reported that lymphocytes from some glomerulonephritis patients showed *in vitro* reactivity against glomerular basement membrane (69), this led to an editorial with the title: “what are sensitized cells doing in glomerulonephritis?” (70). This occurred because the existing view was that humoral immunity was responsible for immune-mediated kidney disease. The immune response can be divided into two general types: innate and adaptive immune responses. The innate immune system consists of cells and proteins that are already present and can be mobilized promptly as a first line of defense to fight pathogens at the site of infection or tissue injury. The innate immune system is comprised of: 1) epithelial cells; 2) phagocytic leukocytes; 3) dendritic cells; 4) natural killer (NK) T cells; and 5) circulating plasma proteins. The adaptive immune system is activated against pathogens that evade the first line of defense. The adaptive immune system is comprised of: 1) a humoral response mediated by B-lymphocytes via antibodies; and 2) cell-mediated by T-lymphocytes. The experimental evidence suggests that accumulation of phagocytic leukocytes and lymphocytes is a constant feature of chronic kidney damage, particularly in the areas of active tubulointerstitial injury and correlates with the severity of renal failure (71-73).

Mononuclear phagocytes in kidney injury and disease

Acute kidney injury (AKI) that is associated with long-term risk for CKD evokes a robust innate immune response. The innate immune response to tissue injury leads to local upregulation of chemokines that facilitate recruitment of neutrophils and naïve monocytes to the site of injury. The microenvironment that is defined by the local milieu at the site of inflammation then promotes maturation of these monocytes to macrophages. Macrophages in turn respond by secreting factors that will either enhance or dampen or simply modulate the inflammation. Several rodent studies indicate that, following recruitment to the site of injury, macrophages are initially activated to a pro-inflammatory state (M1 phenotype) by factors derived from either pathogens, pathogen-associated molecular patterns (PAMPs) or from injured cells themselves called danger-associated molecular patterns (DAMPs). Both

PAMPs and DAMPs are recognized by pattern-recognition receptors (PRRs) such as Toll-like receptors (TLRs) and activate signaling via nuclear factor kappa B (NF- κ B) inducing pro-inflammatory cytokines such as IL-1, IL-6 and TNF- α (74). One major role of M1 type macrophages is to clear out the apoptotic cells or necrotic debris and prep the injured area for repair. Tissue repair is carried out by alternatively activated anti-inflammatory M2 type macrophages and these can be further categorized into wound healing or immunoregulatory subtypes (discussed in detail in (75)). However, macrophages are highly plastic and can trans-differentiate in response to the tissue microenvironment (76-78). In a mouse ischemia-reperfusion model of AKI, Lee and co-workers depleted macrophages in the initial phase of renal injury and determined that M1 type macrophages contribute to tubular injury. Conversely, when macrophages were depleted during the repair phase, the tissue injury worsened. If M2 macrophages were injected in the initial injury phase, lesser tubular injury was observed (79-81). Similarly, studies in the CCR2 (receptor for MCP-1, the major chemoattractants for circulating monocytes) knockout mice determined that CCR2-dependent macrophage recruitment was critical for promoting tubular injury (80, 81). Lee and co-workers made an important discovery that M2-macrophages provided reparative support by promoting proliferation of the surviving tubular cells (79). In contrast, interferon gamma activated macrophages (M1) did not induce tubular cell proliferation. What is also important to note that when M1 activated macrophages were injected into injured mice, these macrophages switched to M2 type. In-vitro studies indicate that factors secreted by tubular cells can induce macrophages to M2 reparative sub-type (79). The observation that M2 macrophages induce tubular cell proliferation provided the rationale for the hypothesis that macrophages in PKD could promote cyst expansion by inducing tubular cell proliferation (discussed below).

Macrophages accumulate in ADPKD kidneys and promote cyst growth

Macrophage-induced proliferation of cells had been proposed almost three decades ago when it was discovered that activated macrophages could induce proliferation in vascular endothelial cells, *in vitro* (82). As mentioned above, macrophages home to injured tubular segments of an ischemically-injured rodent kidney in response to factors like MCP-1, IL-6 and stromal cell-derived factor-1 (SDF-1) secreted by injured tubular or endothelial cells (83). Mononuclear cell infiltrates are present in human PKD kidneys (84, 85) likely due to upregulated MCP-1 (59, 61). Abundance of genes associated with the innate immune response was found in a model of recessive PKD. Indeed most of the markers were related to alternatively-activated macrophages (63). Using an orthologous model of PKD1, C57Bl6 *Pkd1^{fl/fl};Pkhdl-Cre* mice, we provided the first evidence that macrophages could augment cyst growth. In these mice, the cysts start developing from postnatal day 10 and the mice start dying because of renal failure, due to massive cyst growth, by postnatal day 24. We first discovered that, compared to *Pkd1^{fl/+};Pkhdl-Cre* mice, the *Pkd1* null mice harbored ~ ten-fold greater number of F4/80⁺CD45⁺CD11c⁻ macrophages, the majority of which lay in close apposition to cyst-lining epithelial cells. A similar observation was made in *Pkd2^{WS25/-}* mice (a model of PKD2). Using quantitative PCR we then determined that PC1 null tubular cells express seven-fold greater amounts of *Mcp1* and five-fold greater chemokine (C-X-C)

ligand-16 (*Cxcl16*). Thus, a necessary chemotactic gradient exists to attract circulating monocytes and lymphocytes to a PKD kidney. Finally, depletion of macrophages by administering liposomal clodronate, that once taken up by phagocytic macrophages induces apoptosis, to the new born *Pkd1^{fl/fl};Pkhdl1-Cre* mice retarded cyst growth, led to decreased cystic index and improved renal function (blood urea nitrogen values) as compared to vehicle-treated *Pkd1^{fl/fl};Pkhdl1-Cre* mice. More importantly, depletion of macrophages correlated with reduced proliferation of cyst-lining cells without affecting the rate of apoptosis. Also, interestingly, the majority of these macrophages were Ly6C^{low}, consistent with the alternatively-activated macrophage phenotype (86). This study therefore provided a proof of concept, that in ADPKD, inflammatory cells such as macrophages can actively participate in cyst growth or expansion by activating proliferation of cyst-lining epithelial cells. Swenson-Fields and co-workers validated our findings in *cpk* mice model of ARPKD (87). Additionally, they demonstrated the presence of M2 macrophages in kidneys of patients with either ADPKD or ARPKD. Furthermore, they went on to demonstrate that renal tubular cells from ADPKD cysts or noncystic kidneys promote differentiation of naïve macrophages to the M2-like phenotype, in culture. Similar to our study, by depleting macrophages in *cpk* mice, they were able to demonstrate that these innate immune cells promote proliferation of cyst-lining cells (87). Together, these two studies provide biological relevance to previous reports of a strong correlation between expression of the monocyte/macrophage marker CD14 and the rate of cystic progression in *cpk* mice. CD14 expression also correlated positively with an increase in kidney volume that reflects cyst expansion (64).

Along similar lines, a more recent study has determined that macrophage migration inhibitory factor (MIF) promotes cyst growth in *Pkd1^{fl/fl};Ksp-Cre* and *Pkd1^{fl/fl};Pkhdl1-Cre* mice, murine orthologous models of ADPKD (88). MIF is expressed by activated T lymphocytes, macrophages/monocytes, endothelial cells, epithelial cells, cells of anterior pituitary, smooth muscle cells and the synovial fibroblasts, suggesting that perhaps it may play multiple context and/or cell-dependent roles (89-94). Indeed, MIF regulates multiple cellular activities by transcriptionally regulating expression of several inflammation-related gene products such as SRC, ERK, mTOR, AMPK, AKT, p53, TNF- α and MCP-1 (95-102), most of which are also activated in PKD. The authors demonstrated that renal tubular cells lacking *Pkd1* had increased expression of MIF as was also the case in ADPKD kidneys and the cyst-lining epithelia of these kidneys. Treating these mutant mice with a small molecule inhibitor of MIF, isoxazoline (ISO-1), delayed cyst growth that correlated significantly with decreased proliferation of cyst-lining cells and improved renal function. In contrast to our data (after macrophage depletion), they observed increased apoptosis as well. The treated mice had remarkably better parenchyma. These data were further validated in the *Pkd1^{fl/fl};Ksp1-Cre* mice lacking MIF wherein fewer macrophages were observed in interstitial and pericystic areas of the double mutant kidneys. The authors determined that MIF is normally secreted by kidney tubular epithelial cells and this is further enhanced by the loss of *Pkd1*. MIF also induced MCP-1 suggesting that MIF could be upstream of the MCP-1 axis and could therefore regulate trafficking of mononuclear cells into ADPKD kidneys. MIF was also detected in the cyst fluids and urine of ADPKD patients. They further determined that MIF could directly induce cell proliferation by activating ERK via Src kinase and

mTOR pathways by controlling phosphorylation of S6, a substrate of mTOR complex-I. In addition, by activating the p53 pathway, MIF seems to enhance apoptosis in *Pkd1* mutant cells but not in the wild type (WT) cells, which is very important, if it were ever to translate into a therapy (88). Thus, MIF seems to give a bigger “bang for the buck” by affecting multiple aspects of cyst growth and could be an attractive therapeutic target. However, one must be cautious and make sure that inhibition of MIF does not induce apoptosis in normal healthy cells after long-term administration. Given that these patients would be on this treatment for decades, long safety studies would be a pre-requisite.

Other infiltrating innate immune cells in PKD

Cell types other than macrophages have been observed in PKD. As such, CD45⁺ and CD4⁺ lymphocytes have been identified in the interstitium of ADPKD patients (84). Similarly, a few animal models of PKD such as *kat^{2j}/kat^{2j}* mice (103), Han:SPRD rats (104) and DBA/2FG-pcy mice (105) have reported the presence of lymphocytes or their markers in the cystic kidneys. The DBA/2FG-pcy mice develop ARPKD. Segmental dilation of the tubules (distal and collecting duct, similar to one seen in a UUO model) is evident at eight weeks of age and the kidneys get infiltrated with macrophages and lymphocytes at later stages (105). The significance of lymphocytes has not been specifically addressed or studied in PKD. However, CD4⁺ T cells, in particular Th2 cells, have been shown to take part in promoting interstitial fibrosis in a rodent UUO model of kidney fibrosis wherein the tubular dilation is the first event to occur, followed by infiltration of immune cells into the kidneys (106-108). It should be noted that as mentioned earlier, PKD and UUO kidneys share a large number of genetic pathways that are upregulated (14, 86, 109). The most likely reason is that in PKD the enlarging cysts compress and obstruct normal healthy tubules, creating a virtual UUO-like situation, as well as microvasculature, which in turn evokes an inflammatory response, similar to that seen after ureteral ligation in the UUO model (14, 109). Depletion of macrophages or CD4⁺ T cells results in better-preserved renal parenchyma and reduced fibrotic response in the UUO model. Lymphocytes also produce cytokines (interferon- γ and TNF- α) following renal injury that can provide further inflammatory insult to the tissue (110-112). Thus, one could envision a similar role for lymphocytes in ADPKD, although more experimental evidence is necessary.

Mast cells in PKD

An acute inflammatory response can co-exist with chronic inflammation due to simultaneous tissue destruction and repair processes. One example is that of the presence of mast cells, although they are typically associated with an allergic response. Mast cells, surrounded by chymase, have been observed in the kidneys of end-stage ADPKD patients (113). Chymase was present in the interstitial fibrotic tissue. Chymase is not only a chemoattractant for inflammatory immune cells but can convert angiotensin I to angiotensin II (AngII) suggesting the potential of generating Ang-II in ADPKD kidneys, independent of angiotensin-converting enzyme (ACE) and could further contribute to the associated hypertension in PKD. Ang-II also acts as a chemoattractant for inflammatory cells and can activate NF- κ B as well as MCP-1

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expression by activating pro-inflammatory genes (114, 115). Interestingly, kidneys of the mice treated with Ang-II showed polycystic appearance along with inflammatory reactions in the tubules as well as glomeruli. Gene expression studies revealed upregulation of Renin-1 (*Ren1*) and peroxisome proliferator-activated receptor (PPAR)- γ in the kidney tissue (116). Besides, adhesion molecules such as ICAM-1 and VCAM were upregulated, further facilitating infiltration of inflammatory cells. Ang-II has also been shown to stimulate formation of reactive oxygen species (ROS) that are known to cause tissue damage. Intrarenal Ang-II levels are also upregulated in the UUO model (117). Thus, mast cells harbor the potential for contributing to the disease progression of ADPKD, at least via chymase and Ang-II production. However, whether they would play a role during early or late stage of ADPKD remains to be determined.

Complement -3 in PKD

Following tissue injury, the renin angiotensin system (RAS) is also activated by complement-3 (C3) that induces partial dedifferentiation of the epithelial cells which then produce renin through induction of liver X receptor-alpha (LXR α). Recent studies have linked complement activation to cystogenesis. Indeed, gene expression analysis of the ARPKD model revealed dominance of complement system factors that can activate macrophages, including the major complement component C3. This was confirmed by increased C3 protein levels in the epithelia of three different rodent models of ARPKD and human ARPKD (63). Transcriptional dysregulation of complement genes has also been reported in the cells that line the cysts in ADPKD (65). These data were further bolstered by human validation by showing the presence of antigenic C3 in the cyst-lining epithelial cells of ADPKD and C3 activation fragments in renal cysts and urine from ADPKD patients (118). The iC3b fragment was one of the most abundant forms of C3 detected in cyst fluid and urine of ADPKD as well as that of ARPKD patients (119). The authors suggested that while various components of the complement system could act via different receptor pathways, the activation fragment iC3b that is a major macrophage antigen-1 (Mac-1) ligand, likely plays a central role in attachment, survival and differentiation of macrophages. This raises the possibility that iC3b might partly be responsible for cystogenic effects of macrophages. Partial evidence in support of cystogenic role of the complement system was provided by a recent study that observed an over-activated alternative complement system in ADPKD. Specifically, screening of the glycoproteome of urine samples of ADPKD patients showed highly-upregulated levels of complement factor B (CFB) and C9 besides serpin peptidase inhibitor and complement 1 inhibitor (SERPING1). The gene expression was validated by increased protein expression of CFB and C9 in cystic kidneys from ADPKD patients (120). However, the levels of those in serum were not altered. More importantly, the authors provided data to support the relevance of complement activation in progression of PKD in two separate rodent models of PKD, *Pkd1*^{-/-} mice and Han:SPRD Cy/+ rats. To determine the role, if any, of complement in the disease progression, these animals were treated with the complement inhibitor, rosmarinic acid (RMA). Similar to the results from macrophage depletion studies, RMA administration in mice reduced the cystic index by ~ 60% that

was accompanied by significantly improved renal function. Similar results were observed in Cy/+ rats. Lower number of inflammatory cells as well as fewer Ki67⁺ cyst-lining cells was observed that was accompanied by reduced interstitial fibrosis. Whether the beneficial effects observed in this study are largely attributable to reduced macrophage burden remains to be determined. In that regards, a recent study that used an inhibitor of MCP-1 synthesis (Bindarit) in *pck* rats, did not show any effect of cyst progression while it did improve proteinuria and renal function. In this particular study, a 40% reduction of macrophages was achieved (121). The lack of improvement in the cystic index could be attributed to the fact that a greater reduction in macrophage numbers may be necessary to achieve any impact on cell proliferation or cyst expansion. Alternatively, it may simply mean that quenching the pathways of macrophage activation is more critical. These aspects will have to be taken into account when considering any therapy targeting macrophages either directly or indirectly. Thus, experimental evidence is gradually emerging that supports the idea that inflammation could play an important, although not the primary, role in cyst progression in PKD. This role may be directly dependent as well as independent of the altered PC1 signaling in PKD.

Mechanisms regulating immune cell infiltration in PKD

For immune cells to traffic to the site of tissue injury, cells must egress from the bone marrow, or may already be circulating such as PBMCs. They then traverse through the blood vessels, attach and crawl through the endothelium at the specific site of injury into the interstitium. A chemotactic gradient must exist for directed migration of these cells and equally important are the endothelial adhesion molecules such as ICAM-1 and VCAM. Once at the site of injury, the local milieu will determine the activation state of these cells, in particular monocytes/ macrophages. The first step has to be up-regulation of a chemoattractant. Using magnetic resonance imaging (MRI) to detect macrophage trafficking in a mouse UUO kidney, we observed that extremely few macrophages traffic to an unobstructed kidney while the UUO kidney gets inundated with these cells (Yuan, Sherman, Karihaloo and co-workers, unpublished observation ; Figure 2). Following UUO, the obstructed kidney up-regulates the expression of chemoattractants such as MCP-1 and SDF1- α (109) that is similar to the response observed following an ischemic renal injury (122, 123). In PKD, an increased expression of chemoattractants is observed too. However, the difference being that inflammatory markers are seen upregulated even before cyst formation is visible, although the latter could be attributed to the limits of detection methods. For example, a cyst that is 400 μ m in diameter is below the limit of current radiological detection (124). Nearly, three-fold increase in urinary MCP-1 level is observed in ADPKD patients with normal serum creatinine and urinary protein excretion (61). However, a recent study in a slowly progressing PKD model of *pcy* mice that have a mutated NPHP3 gene detected an increase in urinary MCP-1 after having detected microalbuminuria (125). In contrast, studies in the *pck* rats model of PKD detected an increased in MCP-1 already at postnatal week five (121), at which point no formal cysts are observed in this model but the tubules are dilated (126).

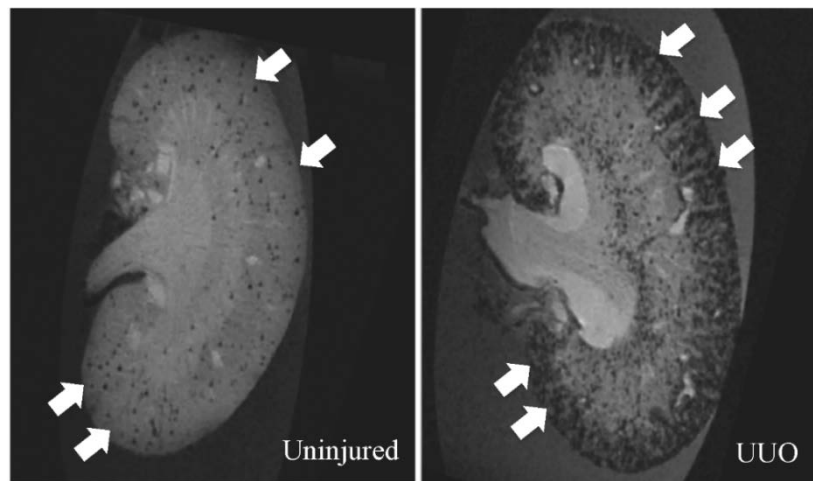


Figure 2. MRI imaging of monocytes tracking to kidney after unilateral ureteral obstruction (UUO). Bone-marrow (BM)-derived monocytes/macrophages were incubated with micron-sized iron oxide particles which they phagocytose. C57/Bl6J mice underwent UUO. 24 hours later, mice were injected these BM-derived iron-oxide containing macrophages and 24 hours after that, mice were sacrificed, kidneys were harvested and processed for MRI imaging. The black dots (white arrow heads) seen in the picture represent the macrophages. Very few macrophages traverse the uninjured kidney while most traffic is to the injured kidney – visible in both cortex and medulla.

Taken together, the aforementioned studies suggest that a change in the expression of a chemoattractant can occur due to multiple stimuli that may be initiated by the cell injury, such as ischemic injury to the kidney, or change in cell-shape as in tubular dilation of UUO or PKD, and/or due to dysfunctional PCs (discussed below).

Polycystin-dependent mechanisms

Others and we have shown that renal tubular epithelial cells lacking PC1 or cells carrying a mutation in *Pkd1* express and secrete significantly higher amounts of MCP-1 *in vitro* and *in vivo*, both in animal models of PKD as well as human ADPKD-derived cells (61, 86, 87). An unbiased systems biology approach for profiling human PKD1 cysts revealed a profile rich in immune and inflammatory responses (65). What this means is that, normally, PC1 would be suppressing any inflammatory responses, and the lack of PC1 or dysfunctional PC1 would release the brakes. Ma and co-workers answered this question when they determined that loss of cilia suppressed cyst growth upon inactivation of polycystins, thereby affirming that PCs are negative regulators of cystogenesis (42). One of the questions then arises, “Does PC1 modulate immune cell trafficking by directly regulating the expression of chemoattractants, such as *Mcp-1*. If yes, how?” While no direct evidence exists to date, we will discuss some potential mechanisms that could support the idea that PCs could directly regulate the expression of chemoattractants.

PC1-C-terminal tail (PC1-CTT) can modulate activity of transcription factors

PC1 undergoes multiple proteolytic cleavages that release both N-terminal extracellular domain and the C-terminal tail (PC1-CTT) (127, 128). To date three cleavages have been reported in the cytoplasmic C-terminal tail of PC1 releasing fragments of different sizes (129-132). The larger 35-kDa fragment accumulates in the nucleus as a result of reduced flow in the rodent kidney (129, 130). It was later determined that PC1-CTT undergoes γ -secretase-dependent cleavage, translocates to the nucleus where it regulates both proliferative and pro-apoptotic signaling pathways by modifying the interactions of transcription factor(s) with the transcriptional co-activator p300 (133). Specifically, it binds to TCF that is activated by β -catenin in response to Wnt ligand to induce- β -catenin pro-proliferative target genes. The binding of PC1 to TCF prevents its interaction with p300 a key transcriptional co-regulator for TCF, thereby acting as an inhibitor of TCF-mediated gene expression and a negative modulator of the Wnt signaling pathway. This 35 kDa PC1-CTT also binds to transcription factor CHOP that partly regulates the apoptotic pathway. CHOP too requires p300 for it to function. PC1-CTT binds to CHOP and prevents its association with p300 thereby reducing its activity. Expressing PC1-CTT in *Pkd1*-null cells provided the biological relevance of these interactions when it rescued increased proliferation and apoptosis of these cells *in vitro*. In the same study, expression of PC1-CTT in zebrafish larvae corrected the dorsal body curvature produced by the loss of PC1 and administration of a γ -secretase inhibitor. Furthermore, using a co-activator trap screen, they determined that several other transcription factors, including pro-apoptotic CHOP-10/GADD153, were significantly regulated by PC1-CTT.

One of those transcription factors is the activating transcription factor 4 (ATF4) (133) that is an ER stress-induced transcription factor. Interestingly, ATF4 stability and transcriptional activity is modulated by p300 (134) and just recently ATF4 was shown to modulate MCP-1 in microvascular endothelial cells (135). This raises an intriguing possibility that PC1-CTT, under a normal state, may negatively regulate MCP-1 expression by potentially preventing the interaction between ATF4 and p300. This needs to be confirmed experimentally. Transcription factor regulator C/EBP β regulates the expression of another macrophage chemoattractant, SDF1- α (136). *Sdf1-a* is upregulated by renal tubules and endothelium of an injured kidney (109). Interestingly, PC1-CTT also interacts with C/EBP β raising the possibility that PC1-CTT can directly regulate yet another chemoattractant. Once again, experimental confirmation has to be provided. Some of these possibilities are summarized in Figure 3.

Polycystin-independent mechanisms

The cyst development begins with focal monoclonal expansion of tubular cells that underwent a second hit. As the cells continue to proliferate, the cyst bulges out as a pouch eventually separating from the tubule (14). These cysts push on the neighboring tubules and microvessels creating a virtual obstructive nephropathy. Simple change of cell shape, such as in a dilated tubule, leads to partial dedifferentiation of the tubular cells as also happens in an obstructed tubule. We recently reported that these partially dedifferentiated cells (in a UUO kidney) acquire semi-mesenchymal-like phenotype and turn on the

expression of *Mcp1*, *Sdf1- α* , *Pdgf- α* , *Tgf- β 1* etc.(109). An innate immune response is triggered to allow macrophages/monocytes home into an injured area directed by the gradient of MCP1/SDF1- α and other chemoattractant molecules. It should be noted that activated macrophages, endothelial cells and vascular smooth muscle cells are capable of producing MCP-1 to reinforce trafficking of additional monocytes/macrophages to the site of inflammation (137). Of note, dedifferentiation of the cyst-lining epithelial cells was also observed in an orthologous *pck* rat model of ARPKD. This was accompanied by an increase in the interstitial fibrosis markers, a hallmark of chronic inflammation (138). These data are further validated by unbiased gene profiling of cysts from PKD1 human polycystic kidneys. PKD1 cysts presented a profile that was rich in genes associated with kidney development, a sign of dedifferentiation/re-differentiation, as well as epithelial-mesenchymal transition (65).

The inflammatory response, once initiated, may feedback upon itself creating a vicious loop. In addition to PC1 dysfunction and/or cell shape change, the complement system is excessively activated in the cyst-lining cells of PKD (discussed earlier) as well in UO tubular cells. C3 activation leads to generation of renin that in turn increases intra-renal Ang II (117). Ang II in turn induces the expression of adhesion molecules including ICAM-1 and VCAM-1 and chemokine MCP-1(116).

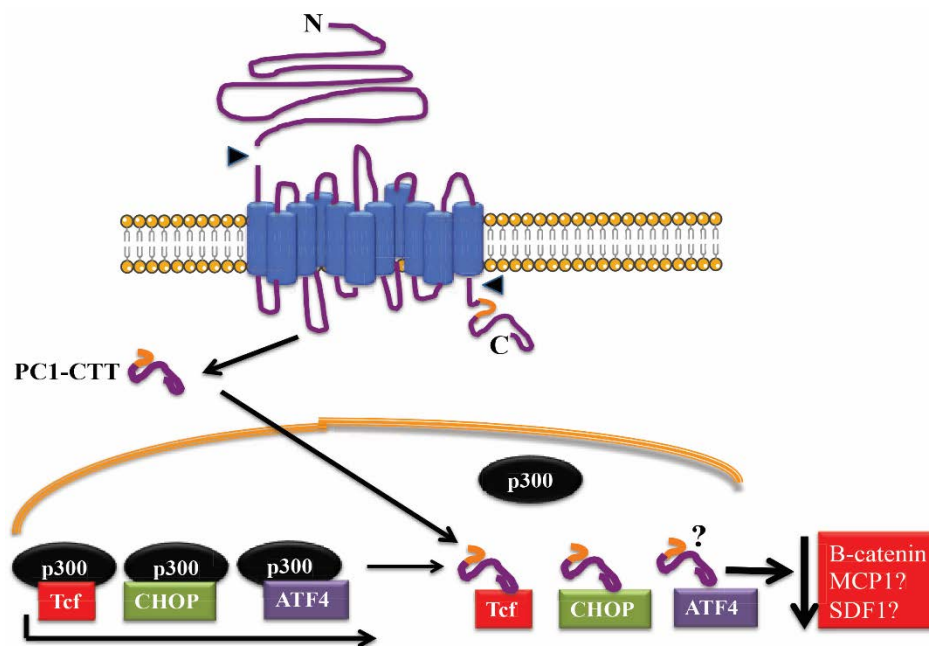


Figure 3. The cartoon shows potential mechanism by which PC-1 may modulate transcription. PC1 undergoes cleavage at the N and C-terminus (indicated by arrow heads). The C-terminal tail (PC1-CTT) translocates to the nucleus where it binds TCF, CHOP (confirmed) and ATF4 (speculated) to control transcript of β -catenin-dependent targets (confirmed) and MCP1 and/or SDF-1 α transcription (speculated).

In addition to the above mentioned mechanisms, tubular cells of a PKD kidney (Hans:SPRD rat) are known to have an increased expression of osteopontin that too harbors monocyte/macrophage chemoattractant properties (139). Thus, immune cells may traffic to a PKD kidney to cues that are generated in a PC1-dependent and/or independent manner.

Macrophage activation

Macrophage activation can be classified into two categories: classically activated M1 and alternatively activated M2. This classification has been largely guided by responses (*in vitro*) to the prototypic T-helper 1 (Th1) cytokine, interferon-gamma (IFN- γ or Th2 cytokine interleukins 4 (IL-4) and 13 (IL-13) (140).

M1 macrophages are proinflammatory first responders whose job is to clear debris and apoptotic cells. Upon ligation with its receptor on macrophages, IFN- γ activates Janus kinase (JAK)1/2 and STAT-1 inducing a proinflammatory battery of chemokines, MCP-1 and monokine induced by gamma interferon (MIG) that together help recruit monocytes and T cells to the site of injury and inflammation (141). However, *in vivo*, in a sterile injury, DAMPs such as adenosine triphosphate (ATP) or nucleic acids that are released by the injured tissue, interact with their pattern-recognition receptors (PRRs) such as TLRs (142) and induce macrophages to a proinflammatory M1 state. Ligation of DAMPs by PRRs activates transcription factors such as NF- κ B, augmenting the levels of proinflammatory cytokines such as TNF- α , IL-1, IL-6 and IL-12 (74, 143). Thus, infiltrating monocytes differentiate into proinflammatory M1 macrophages via activation of STAT1 and TLR/NF- κ B pathways. As mentioned earlier, M1 macrophages can exacerbate tissue injury (79). M1 macrophages are highly phagocytic and therefore help clear apoptotic cells and cell debris as an obligatory step to initiate tissue repair by M2 macrophages.

M2 macrophages hold a very diverse functional portfolio. They participate in wound healing but can also promote fibrosis and can be identified by IL4-induced expression of arginase-I (ARG1), mannose receptor (MR), Ym1 and insulin-like growth factor -1 (IGF-1), reviewed elsewhere (144). IL-4 (as well as IL-13) signal through IL-4R α and activate the STAT6 pathway that in turn regulates ARG1 and Ym1. Interestingly, STAT6 engages with transcriptional co-activator such as C/EBP- β (135, 145). Yet another study indicates that p300/CBP too interacts with STAT6 and is necessary for IL-4-induced transcription of STAT6 (146). This raises the possibility that under healthy conditions, PC1-CTT while interacting with p300 could suppress STAT6 expression or signaling, similar to suppressing β -catenin-mediated transcription, and in the setting of loss of PKD1/2 or dysfunctional PC1/2, the inhibitory effect would be lost resulting in an overexpression/activation of STAT6. Indeed, Olsan and co-workers have demonstrated increased expression of STAT6 in cyst-lining epithelial cells in mouse PKD models (147). They also reported the presence of IL-13 (ligand) in cyst fluid and the overexpression of IL-4/13 receptor that would result in sustained activation of STAT6. Ablation and inhibition of STAT6 led to blunting of cystic expansion (147). However, macrophage accumulation or activation was not evaluated in

this study. One must also keep in mind that the experimental evidence also suggests STAT6-independent mechanisms for alternative macrophage activation (79).

As described above, M1 macrophages switch to the anti-inflammatory M2 pro-repair or pro-fibrotic phenotype. The kidney epithelial-derived factors that can facilitate this switch are not, as yet, very well defined. However, colony stimulating factor-1 (CSF1) is one factor that has been implicated in the switch, at least in part. More recently, granulocyte-macrophage (GM)-CSF was demonstrated to induce alternative activation of macrophages, following ischemia-reperfusion injury (148). Swenson-Fields and co-workers demonstrated that epithelial cells from human ADPKD cysts (as well as conditioned medium from these cells) promote distinct M2 differentiation of naïve as well as polarized macrophages and this effect was more potent than non-cystic tubular cells (87). These data provide a proof of concept that cyst-lining ADPKD cells must secrete soluble factor(s) that have the potential for inducing naïve or polarized macrophages to an alternative phenotype that is pro-repair but also pro-fibrotic.

Thus, there is reasonable evidence to suggest a direct role of PC1 in regulating chemokine/cytokine expression that facilitates the trafficking of innate immune cells to the cyst-lining cells in a polycystic kidney. There is sufficient evidence documenting the abundance of M2 type macrophages in ADPKD. There is also evidence that soluble factors derived from ADPKD cyst-lining cells can induce naïve or already polarized macrophages to M2, pro-repair/pro-fibrotic phenotype. This is very similar to what has been described in an ischemia-reperfusion model (discussed earlier) where M1 macrophages switched to an M2 activation state to facilitate repair by inducing tubular cell proliferation (79). However, the questions that have not yet been answered are: "What other factors are responsible for the macrophage phenotypic switch and the tubular cell proliferative response? Does PC1 directly modulate their expression in any way"? While currently we do not have answers to these questions, there are data demonstrating transcriptional regulatory properties of PC1, which raises the potential possibility that PC1 could indeed regulate the expression of some of those (as yet undefined) factors. Of note, ~15 kDa and ~30kDa PC1-CIT fragments have been found to accumulate in ADPKD patient kidneys. These complexes regulate transcriptional pathways and the activation of STATs as we described above (132). We now discuss evidence that suggests PC1-mediated STAT activation in non-lymphoid cells.

PC1 regulates activity of STATs in non-lymphoid cells

The STAT family of transcription factors is activated by JAK and plays a role in modulating immune responses, cell survival and differentiation. Activation of STATs leads to their binding to phospho-tyrosine residues of the receptors of activated growth factors or cytokines followed by STAT phosphorylation via receptor tyrosine kinases such as JAK or non-receptor tyrosine kinase like Src. Soon after, nuclear translocation follows where STATs carry out their diverse transcriptional regulatory functions (149). Of the six known STAT family members, only STAT3 and STAT6 have been shown to play some role in cyst progression of ADPKD.

The very first evidence of modulation of STAT activity by PC1 emerged when it was shown that overexpression of PC1 binds JAK2, activates STAT1 and induces cell-cycle arrest by up-regulating p21 (waf1) (150). Subsequently, Low and co-workers discovered that PC1-CTT undergoes cleavage, releases a ~ 17kDa PC1-CTT fragment that translocates to the nucleus, interacts with STAT6 transcriptional co-regulator p100, and co-activates STAT6-dependent gene expression (131). The same group later determined that PC1 activates STAT3 (132) that is in part dependent on Src. It was determined that membrane-anchored, full-length PC1 binds JAK2 and activates STAT1 and STAT3, and the cleaved CTT co-activates STAT1, STAT3 and /or STAT6 (151). It is important to note that cleaved CTT by itself does not activate STATs but can co-activate previously activated STAT, such as by a specific growth factor, thereby amplifying the signal.

Both, STAT3 and STAT6 are aberrantly activated in cyst-lining cells of rodent PKD models. Pharmacological inhibition of STAT3 or STAT6 and genetic ablation of STAT6 slows down the cyst growth in these PKD models (147, 152, 153). These studies clearly highlight the significance of these two transcription factors in PKD. However, eventually their clinical significance will be judged by their relevance to human PKD patient population. For detailed description of the role of PC1 in modulating transcription the readers are directed to (154, 155). It should be noted that global gene analysis on human PKD1 renal cysts has revealed that, of the 100 most upregulated genes, 11 were associated with the JAK-STAT pathway while three were linked to the NF- κ B pathway (65).

NF- κ B and PKD

NF- κ B is a complex of proteins that, in response to stress such as free radicals, controls cytokine production and cell survival. Aberrant NF- κ B regulation is linked to inflammatory and autoimmune diseases and can be activated by stimulation of TLRs (156). Generally, NF- κ B is associated with the acute phase of inflammation wherein upon stimulation, the p65 subunit (normally located in cytoplasm) of the NF- κ B complex gets phosphorylated, translocates to the nucleus and initiates transcription (157). The cytokines regulated by NF- κ B include TNF- α , IL-1 α and β , IL-6, Ccl3, Ccl4 and MCP-1 (158). Of these, TNF- α , and IL-1 β can also activate the NF- κ B pathway, thereby creating a self-perpetuating feedback loop (159). A few studies have demonstrated the importance of NF- κ B pathway in PKD, discussed below.

It was discovered that cells lacking *Pkd1* have higher amounts of phosphorylated p65 that indicates activated NF- κ B (160). Subsequently, phosphorylated NF- κ B protein was detected in the nuclei of cyst-lining cells in a mouse PKD2 model as well as in human ADPKD kidneys (161). Coincidentally, the receptors of advanced glycation end product (RAGE) and s100a8/9 levels were found to be elevated in PKD2 mice (161). RAGE is a mediator of NF- κ B and is activated not only in CKD (such as in diabetic nephropathy) but in neuroinflammation as well (162). Recently, Qin and co-workers reported that hyperactivated c-Met (the hepatocyte growth factor/HGF receptor) in mice lacking *Pkd1* led to increased NF- κ B signaling which in turn upregulated the expression of Wnt7a and

Wnt7b that are involved in cell proliferation. Inhibiting the Wnts led to a decrease in cystic area (160). TNF- α , a target gene of the NF- κ B pathway, induced cyst growth in explanted kidneys in Pkd2^{+/-} and wild type kidneys suggesting that TNF- α can directly incite cystogenesis (60). Together, these data provide some evidence that support a role for NF- κ B in ADPKD. Further studies would have to be conducted to firmly establish a role for this pathway in the progression of ADPKD.

Significance of chronic inflammation in PKD

There is a wide variability in the rate of decline in renal function and progression to ADPKD. While it is undoubtedly influenced by the specific mutation, gene dosage and epigenetics, sustained chronic inflammation could be yet another factor influencing the rate of progression of ADPKD. Inflammation seems to correlate with disease initiation and progression, given that it is present even before any noticeable functional alteration and continues to be present in the late stages as well. Data discussed above suggest that inflammation may even promote cystogenesis via its effector cellular components, such as macrophages, and a complementary activation of certain signaling cascades, such as NF- κ B and TNF- α . There is also some evidence in the literature that suggests targeting inflammation may have beneficial effects in the PKD patient population. For example, drugs that are typically used for controlling hypertension, angiotensin-converting-enzyme inhibitors (ACEi) and angiotensin-II receptor blockers (ARBs) may have anti-inflammatory benefits as well. They have been shown to lower markers of inflammation in hypertensive ADPKD patients (163). Similarly, an improved renal function correlates with reduced inflammatory cell infiltrate (86, 87). It will be interesting to find out whether a nearly 50% reduced kidney growth rate (18) in Tolvaptan-treated patients translates to corresponding lesser inflammation as well. A list of compounds with potential anti-inflammatory effects has been provided by Ta and co-workers elsewhere (10).

An additional factor to be considered is interstitial fibrosis that goes hand in hand with inflammation. Indeed, fibrosis is a hallmark of chronic inflammation. While it is beyond the scope of this chapter to discuss the potential role of fibrosis in ADPKD, there is no doubt that fibrosis is detrimental to organ function. Indeed, tubulointerstitial fibrosis and not the glomerular lesions was found to be a significant predictor of renal prognosis (164) in type II diabetics. The potential role of fibrosis in PKD is described elsewhere (165).

Conclusion

Taken together, the current evidence suggests that inflammation begins very early in PKD, potentially even before any overt cyst growth, and seems to correlate with the progression of disease. Undoubtedly, the underlying mutation and the resultant dysfunctional PC1/2 is the basic cause for cystogenesis but inflammation could play

a very important role in cyst progression. This is supported by three experimental studies where a decrease in macrophage content led to an improved parenchyma, reduced cyst burden and improved renal function (86-88). However, targeting inflammation directly for therapeutic purpose in PKD (and CKD in general) poses challenges. For example, both active and chronic inflammatory pathways are activated concurrently, particularly in the late stages of PKD. Hence, one will have to maintain a fine balance between the good and the bad inflammation, knowing that, inflammation after all, is a friend as well.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 15

Autosomal Dominant Polycystic Kidney Disease Induced by Ciliary Defects

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is a common genetic renal disease, which is caused by pathogenic mutations of either PKD1 (85%) or PKD2 (15%) genes, encoding for polycystin-1 (PC1) or polycystin-2 (PC2), respectively. These two proteins hetero-dimerize in renal primary cilia to act as a calcium channel. Primary cilia that protrude from cell membranes have a microtubule-based finger-like structure and are found on most mammalian cells. Primary cilia in the kidney have no motility but act as mechanosensors to sense fluid flow through renal tubules. In addition, various signaling

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proteins related to Hedgehog (Hh) and platelet-derived growth factor receptor alpha (PDGFR α) are localized to the cilia to detect changes in the extracellular environment. Recent studies have demonstrated that many ADPKD animal models have defective cilia in the epithelial cells that line the cysts. Also, animal models targeting ciliary genes show abnormal phenotypes such as polycystic kidneys and developmental defects. These findings reveal that ciliary malfunction is sufficient to cause ADPKD. In this chapter, we will review the putative roles of cilia in cyst formation and development in ADPKD.

Key words: ADPKD; Cilia; Ciliopathies; PKD1; PKD2

Introduction

Polycystic kidney disease (PKD) is a group of inherited kidney disorders that induce bilateral cyst development in the kidneys. PKD is classified into two types: autosomal dominant (AD) and autosomal recessive (AR). ADPKD is estimated to have a prevalence rate of 1:400-1:1000 worldwide (1). ARPKD is estimated to have a prevalence rate of 1:10,000-1:20,000 (2). ADPKD is the most common case of PKD and occurs in middle age, whereas ARPKD is the most lethal form and even affects children (2). One of the prominent characteristics of ADPKD is the development of fluid-filled cysts induced by an abnormal cell proliferation of epithelial cells in both kidneys, followed by inflammation and fibrosis leading to chronic kidney failure. Despite the clinical significance of this disease, no effective treatments are currently available. Mutations in the polycystin genes, PKD1 and PKD2, are responsible for ADPKD. These proteins are located in the primary cilia of tubular cells. The discovery of several mutated proteins in human and murine ADPKD indicates that there is a tight correlation between primary cilia and cyst formation, cell polarity, STAT6 and mammalian target of rapamycin (mTOR) signaling (3). Understanding the relationship between ADPKD pathogenesis and ciliary defects will provide novel insights to develop specific therapeutic targets against ADPKD.

Autosomal dominant polycystic kidney disease (ADPKD)

ADPKD is the fourth leading cause of renal failure worldwide in adults and affects approximately 1 in 400 to 1 in 1000 people (1, 4). ADPKD is a multisystem disease characterized by numerous cysts and fluid secretions into the lumen in the bilateral kidney (5). In general, patients affected with ADPKD suffer from hypertension and other cardiovascular symptoms beginning in their twenties and grow lots of fluid-filled cysts by middle age, finally leading to end-stage renal disease (ESRD) in ~50% of cases, which requires dialysis or transplantation (4).

The pathophysiology of ADPKD is caused by mutations in the genes of PKD1 (chromosome region 16p13.3; approximately 85% of cases) or PKD2 (4q21; approximately 15% of cases), which encode the proteins polycystin-1 (PC1) and polycystic-2 (PC2), respectively. Mutation in PKD1 is associated with a more severe renal cystic disease than mutations in PKD2 (6). PC1 is a 450-kD protein with a large extracellular N terminus, 11 membrane-spanning domains, and a shorter cytoplasmic C terminus (7) and is associated with cell-cell and cell-matrix interactions at tight junctions, adhesions junctions, desmosomes, and focal adhesions (8). PC2 is a 968-amino acid protein that has six transmembrane domains with intracellular N and C termini (9). PC1 and PC2 proteins are known to form a complex that plays a role as a transient receptor potential channel involved in the regulation of intracellular calcium homeostasis (10, 11). This complex is localized to the primary cilium (12) and the endoplasmic reticulum (ER) (13), where it affects calcium concentrations in several subcellular compartments (14, 15). In the primary cilium, the PC1-PC2 complex may play a role as a mechanoreceptor to induce the influx of extracellular calcium in response to fluid shear stress (16, 17), while in the ER, it interacts with the ryanodine receptor and plays a role as a calcium release channel (18). Although the mechanisms are unclear, the loss of the functional PC1-PC2 complex leads to phenotype alterations such as the inability to maintain planar cell polarity, an imbalance between cell proliferation and apoptosis, increased fluid secretion, and remodeling of the extracellular matrix. The major signaling pathways associated with these phenotypic alterations include the intracellular deregulation of calcium homeostasis, cAMP accumulation and activation of protein kinase A (PKA), activation of mitogen-activated protein and mammalian target of rapamycin (mTOR) kinases, canonical Wnt signaling, and other intracellular signaling mechanisms (19, 20).

The most important abnormalities that occur in the tubular epithelium lining the cysts have been extensively described: disturbance in the balance between tubular cell proliferation and apoptosis, alterations in the polarity of membrane proteins, abnormalities of cell-matrix interactions, abnormal fluid secretion, and abnormal ciliary function (21).

At first, in ADPKD, abnormal proliferation in tubular epithelial cells is strongly associated with cyst development and/or growth. The process of cyst formation requires proliferative expansion of the epithelial lining of the collecting duct or renal tubules (22). Actually, increased proliferation was observed in early cysts or dilated tubules from human ADPKD specimens and some mouse models of sporadic ADPKD (23, 24). Increased apoptosis as well as cell proliferation is detected in kidney tissues with ADPKD. Although the precise pathways linking proliferation and apoptosis in ADPKD remain to be elucidated, there is some evidence that apoptosis plays a crucial role in cystogenesis: (1) tubular epithelial cell apoptosis is observed in most animal models of

PKD and in kidneys from patients with ADPKD; (2) induction of apoptosis in renal tubular cells leads to cyst formation *in vitro*; (3) abnormal increase in both proliferation and apoptosis occurs in cystic and non-cystic epithelial cells in the early stages of ADPKD; (4) caspase inhibition may induce less proliferation and apoptosis in tubular epithelial cells, leading to reduced cyst formation and kidney failure (25). Intriguingly, dysregulation of apoptosis plausibly induces cystic remodeling of renal tissue in cooperation with increased proliferation of tubular cells with disrupted planar cell polarity (PCP) and disoriented mitotic spindles (26).

The PCP pathway, which is necessary for oriented cell division and the establishment/maintenance of kidney tubule structure, is involved in ADPKD pathogenesis even though it is incompletely understood (27). In addition, cell-cell/cell-matrix interactions, which are mediated by integrin receptors, have long been associated with ADPKD but remain firmly understudied. Overexpression of extracellular matrix (ECM) proteins has been observed in human ADPKD cells and ADPKD animal models. In addition, the cysts lining epithelial cells show elevated adhesiveness to type I and type IV collagen in response to growth factors (28).

Fluid secretion is an important pathogenic mechanism of cyst development in ADPKD. A large number of cystic lesions exhibit loss of afferent and efferent tubule connections, which implies that cysts derived from tubular segments are disconnected from the glomerular filtrate. Therefore, net transepithelial fluid secretion is required for the expansion of cystic lesions (29). Fluid accumulation causes cyst enlargement due to swelling and stretching in the cells to stimulate cellular division (30). Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-regulated chloride channel expressed in the apical membranes of many secretory epithelia.

Finally, renal cilia are microtubule-based and play a role as mechanosensors in response to fluid flow. The mechanosensory function of cilia is lost with mutated PC1 proteins in renal tubular cells (16) and the loss of polycystin function is mostly linked with cilia, leading to abnormal calcium signals in response to fluid flow (3).

Primary cilia

Structure of cilia

Cilia and flagella are hair-like organelles found on eukaryotic cells when the cells were growth arrested or differentiated. These organelles project from the apical membrane of

epithelial cells. Although cilia were discovered in the 17th century, only motile cilia were studied for a long time, for example, in the respiratory epithelia that mediate airway clearance (3, 31). Relatively recent studies have focused on the structure and functions of primary cilia.

Cilia and flagella are identified by their structures. Although the outer membranes of cilia and flagella are lipid bilayer membranes that coincide with the plasma membrane of the cell body, receptors and other proteins involved in signaling are embedded in the outer membrane of cilia (32). The inside structures of cilia and flagella are comprised of a microtubule-based cytoskeleton known as axoneme, which is a cylindrical pole regulated by the assembly or disassembly of ciliary protein. The axoneme grows outward from the basal body, which is a modified form of centrioles for developing axonemes in cell-cycle arrested cells. The centrosome is a complex of two centrioles and functions as the main microtubule-organizing center (MTOC) (33). The core axoneme is comprised of nine outer doublet microtubules (9+0) that emanate from the triplet microtubules of the mother centriole in the basal body (34). The change in the microtubule structures occurs in the region where the microtubule attaches to the membrane, known as the transition zone. The transition zone has transitional fibers that emerge from the end of the basal body and function as linkers from the doublet microtubules to the ciliary membrane (35, 36). The ciliary or outer membrane particles are separated in the transition zone. Selected ciliary particles move to the ciliary compartment and the membrane associated protein particles are lined up in the region known as the 'ciliary necklace' (37, 38).

Most motile cilia contain an additional pair of central microtubules and axoneme-associated dynein arms as well as radial spokes for ciliary motility (36). Recent studies, however, found some motile cilia with '9+0' or '9+4' microtubule structures (39). The non-motile cilia also known as 'primary cilia' are comprised of nine outer doublet microtubules, but lack a pair of microtubules and other proteins involved in motility (Figure 1). Instead of motility, primary cilia function as 'sensory antennas'. The ciliary membrane contains a subset of receptors and ion channels that induce primary ciliary signaling pathways including phototransduction, olfactory sensing, mechanosensing, extracellular signaling including Hedgehog (Hh), Wnt, Platelet derived growth factor (PDGF) ligand, and planar cell polarity (PCP). Many organs and tissues in the mammalian body such as the brain, kidney, liver, pancreas and oviduct as well as olfactory and visual organs also have non-motile, primary cilia that detect and transmit signals from the external environment (32, 40, 41).

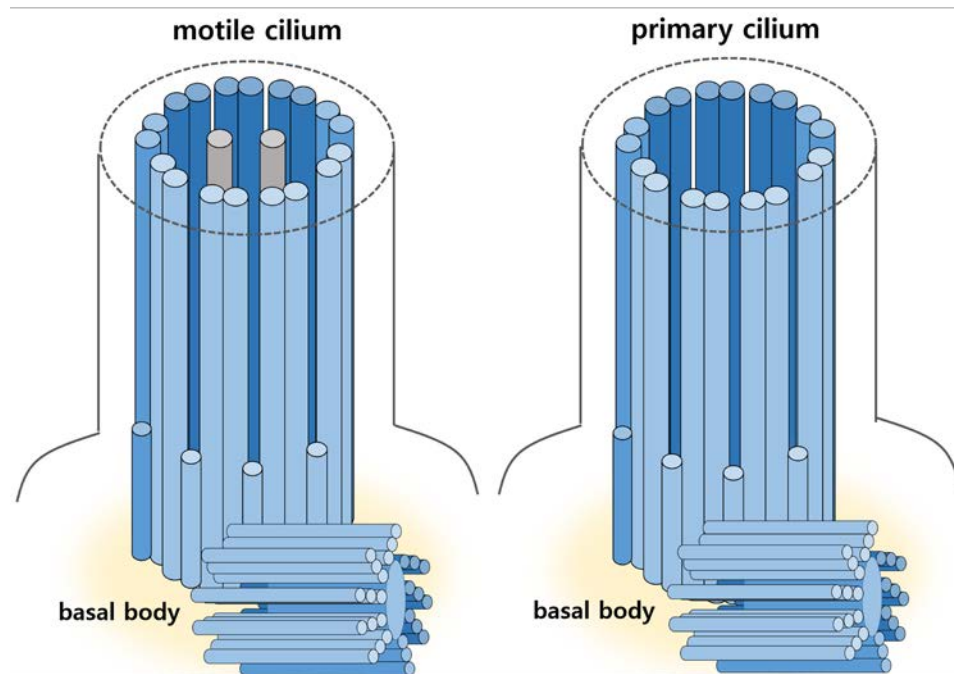


Figure 1. Structure of motile cilia and primary cilia. The inside structure of motile cilia is 9+2, and primary cilia is 9+0 microtubules based on the cytoskeleton, called the axoneme, which is a cylindrical pole regulated by the assembly or disassembly of ciliary protein. The doublet microtubule emanates from the basal body, which is a modified form of centrioles in cell-cycle arrested cells, ultimately resulting in assembly of the cilia.

Cilia and cell cycle

The cilia originate from the triplet microtubules of the basal body during interphase of cell division. The basal body is known as the centriole in metaphase of cell division. The centriole plays a role in determining the position of a dense matrix, called pericentriolar material, which in turn functions in organization of the microtubule during cell division (42). Because of basal body (centriole), formation of primary cilia also closely related with cell cycle regulation. Cilia are resorbed before S phase or during G2 (43) (Figure 2). When Golgi-derived (primary) vesicle attach to the mother centriole in the phase of G1, assembly of primary cilia begins. Additional Golgi-vesicles transport axonemal subunits at the mother centriole, and then accessory structures that induce docking and attachment of the mother centriole to the apical plasma membrane are formed (44, 45). Since docking of the mother centriole, axonemal subunits add to ciliary axoneme, and it leads to assemble and elongate primary cilia (35).

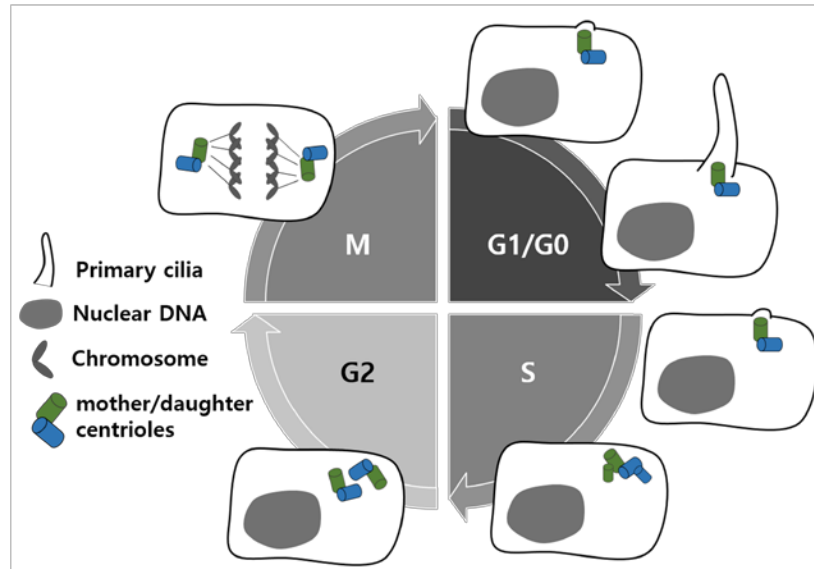


Figure 2. Regulation of primary cilia formation during the cell cycle progression. During the G1 phase, centrioles dock to the apical region of the cells to prepare for cilia formation. If cells enter the G0 phase, assembly of the primary cilia is induced. During the S/G2 phase, centriole duplication and cilia absorption/disassembly occur, leading to cell division. When progression of the cell cycle is complete, two daughter cells re-enter the G1 phase and prepare for cilia re-assembly.

Assembly of cilia

A number of components for cilia assembly have to be transported by ciliary precursors. Ciliary precursors were not described until 20 years ago, however, intraflagellar transport (IFT) was first discovered in *Chlamydomonas*, a unicellular green alga, by Rosenbaum and his colleagues (35, 46). They observed that IFT ascended and descended between the distal end of the flagella and the basal body along the ciliary axoneme (46). IFT was induced by large protein complexes known as complex A and B according to the direction of movement. Particles of IFT complex B function in anterograde IFT to the distal end of the cilia for ciliary assembly, whereas particles of IFT complex A are associated with retrograde IFT to the basal body during ciliary disassembly (47, 48). Anterograde or retrograde movement requires two classes of motor proteins, kinesins and dyneins, which gain energy by ATP hydrolysis. Heterodimeric kinesin-2 motors (Kif3a, Kif3b, KAP complex in mammals) accumulate IFT complex B (anterograde IFT) particles and transport them toward the tip of the cilia. Cytoplasmic dynein 1b (DHC2 in mammals) carries IFT complex

A particles (retrograde IFT) from the tip to the base (49-51). Recent studies reported that kinesin-2 motors congregate with not only IFT particles but also associated cargoes such as axonemes precursors, signaling molecules and retrograde motors, resulting in anterograde assembly in the cilia. Cytoplasmic dyneins, in contrast, restore kinesin motor proteins and IFT particles to the basal body (34).

Ciliopathies

Cilia were previously regarded as no more than small organelles. Following increased interest in cilia, however, studies have focused on disruptions in the cilia. Ciliary dysfunctions are related to multiple human genetic diseases called ciliopathies (52). The first study observed some developmental defects in Oak Ridge Polycystic Kidney mouse (ORPK mouse, mutation in IFT88) including cystic kidneys (32, 53). Later studies demonstrated that IFT88 was responsible for the assembly of the cilia and for abnormal cilia in the Tg737/ift88 mutant mouse (32, 42, 54). The importance of cilia in renal cystogenesis was revealed by knock-down of KIF3a (IFT-associated kinesin motor) in mouse kidneys (55). Altogether, loss of cilia or polycystin, which disrupted ciliary signaling, resulted in cystic disease. Recent data indicated that the timing of cilia defects determine the severity of the cystogenetic phenotype (56).

PKD and ciliary defects

Among the known ciliopathies, PKD is one of the most common renal genetic disorders. Recent studies have demonstrated that mutations in ciliary genes are closely related to the onset of cystic kidneys (12). Therefore, cilia-defective mouse models were produced to elucidate the function of renal cilia in the kidney. Most mouse models targeted by ciliary proteins showed embryonic lethality with multiple developmental defects (57-59). Consequently, many research groups have produced ciliary gene-targeted mice using kidney specific Cre mice (54, 57, 60). In this chapter, the phenotypes of renal cilia and aberrant signaling pathways in PKD mouse models targeted by ciliary genes are introduced.

Phenotype of renal cilia in representative PKD mouse models

According to many papers published so far, proteins related to the onset of PKD are localized to the cilia or basal body and regulate ciliary functions as well as the structure of the cilia (12, 61). The PC1 and PC2 proteins, encoded respectively by PKD1 and PKD2 genes, are localized to renal cilia. DeCaen et al. suggest that these two protein complexes

act as calcium channels in the primary cilia (62, 63) and regulate various intracellular signaling pathways associated with cell proliferation (64). Kidney-specific inactivation of *Pkd1* in the mouse results in the severe polycystic kidney phenotype (65), but there are no significant changes in the renal cilia (56, 65). However, a knockin mouse model targeted by pathogenic mutation of PKD1 (PKD1 p.R3277C) shows progressive PKD phenotype with elongated cilia of the renal collecting duct cells (66). These results indicate that the function and structure of renal cilia are regulated according to the type of genetic mutation of PKD1. The first mouse model to demonstrate that defects in renal cilia are associated with the development of PKD was the ORPK mouse model. The ORPK mouse is produced by insertion mutation of the *Ift88* (*Tg737*) gene related to ciliary assembly (54). This mouse model has the polycystic kidney phenotype with shortened renal cilia that accumulate with PC2 protein (53, 67). As the volume of papers on the relevance of ciliary defects and PKD has increased, various mouse models targeted by ciliary genes have been produced.

Many research groups have proposed that deficiency in the renal cilia is the driving force for PKD development. In this section, representative PKD mouse models targeted by the IFT complex B or complex A subunits are described. Most mouse models that are constitutively targeted by genes related to IFT have shown embryonic lethality (55, 57), and as a result, mouse models that are specifically targeted to IFT genes were produced to identify the role of IFT genes in the kidney. In PKD mouse models targeted by the IFT complex B subunit, deletion of the *Ift20* gene in the collecting duct cells of the kidney is well documented. Because *Ift20* belongs to IFT complex B, which has a role in cilia assembly (57), inactivation of *Ift20* gene may cause defects in cilia formation. As expected, polycystic kidney, accompanied by the absence of cilia and centrosome defects such as mislocalization and overduplication leading to misorientation of the mitotic spindle, is observed in the *Ift20* targeted renal collecting duct cells (57). In the PKD mouse model targeted by the IFT complex A subunit, inactivation of the *Ift140* gene in the collecting duct cells of the kidney is well documented. This mouse model exhibits severe polycystic kidneys accompanied by an increase in canonical Wnt signaling (60). However, the renal cilia phenotype for *Ift140*-deleted kidneys is slightly different from those of *Ift20*-deleted kidneys. Renal cilia are almost completely absent in *Ift20*-deleted kidneys, but inactivation of *Ift140* in the kidney results in short or stumpy cilia despite cystic renal epithelial cells in the late stage (60). These studies indicate that defects in IFT complex B commonly induce loss of renal cilia while defects in IFT complex A appear to induce short or truncated renal cilia instead of lack of cilia, suggesting that normal renal cilia structure is critical for repressing the cystogenesis mechanism in PKD.

Another PKD mouse model targeted by mutation in non-IFT genes is the juvenile cystic kidney (*jck*) mouse, which has a missense mutation in the *Nek8* gene encoding

serine/threonine kinase, NIMA (never in mitosis A)-related kinase 8 (68). The NIMA protein is known as the mitosis regulator and controls cell cycle entry (69). It has been suggested that cell cycle regulators may be involved in the regulation of primary cilia structures because primary cilia are absorbed into the cells during the cell cycle, resulting in disassembly of the primary cilia (70). Therefore some regulators related to cell cycle progression, such as the NIMA protein, may have an effect on the primary cilia structure. Consistent with this prediction, lengthened renal cilia are observed in the kidneys of *jck* mice with the accumulation of PC1 and PC2 expression along the cilia (71). With these ciliary defects, the *jck* mouse model shows the PKD phenotype in multiple nephron segments with increased levels of cAMP, resulting in an increase in fluid secretion into the lumen and renal cell proliferation (71).

These PKD mouse models showing defects in renal cilia suggest that ciliary defects, including normal cilia without polycystin, stumpy cilia, and lack of cilia, are a driving force in the development of cystic kidney, and that cilia are important to maintain normal physiology in the kidney. Therefore, understanding the pathological changes that are specifically influenced by renal ciliary defects is important to elucidate the mechanism underlying renal cystogenesis.

Aberrant signaling pathway induced by ciliary defects in PKD

Unlike motility cilia, primary cilia have no motility but are considered cellular antenna that transduce extracellular environmental changes to intracellular signaling molecules (72), suggesting that defects to the cilia lead to aberrant multi-signaling pathways. In PKD with ciliary defects, many signaling pathways related to cell proliferation are disrupted (73). Among the pathways disrupted in PKD, mitogen-activated protein kinase (MAPK) and mammalian target of rapamycin (mTOR) pathways are commonly activated in PKD (74, 75).

The primary cilium in the renal epithelial cell protrudes from the plasma membrane into the lumen to sense flow stimulation through the renal tubules (16). Flow stimulation induces the bending of intact cilia with PC1/PC2 calcium channels (16, 76), which leads to an increase in intracellular calcium levels followed by the release of calcium from the endoplasmic reticulum (ER). Increased levels of intracellular calcium induce suppression of the Ras/Raf/Mek/Erk pathway by regulating cAMP (42, 77). In contrast, perturbation of flow sensing occurred in PKD in the absence of cilia, resulting in the decrease of intracellular calcium levels (78). Decreased intracellular calcium levels induce cAMP activation, leading to activation of the Ras/Raf/Mek/Erk pathway. Hence, increased cell proliferation and fluid secretion into the lumen are observed in many PKD models with cilia defects (79).

The mTOR signaling pathway plays a role in regulating cell size and metabolism (80). Accumulated data suggest that hyper-activation of the mTOR pathway is observed in various PKD mouse models (75, 81). Based on these data, many research groups have tried to find the regulatory mechanism of the mTOR pathway in PKD models with cilia defects. There is a paper elucidating the role of renal cilia in regulation the mTOR pathway (82). This paper suggests that Lkb1 and AMPK proteins, localized at the basal body of normal primary cilia, repress the mTOR signaling pathway under flow conditions to reduce cell size (82). However, enlarged cell size and hyper-activation of mTOR signaling are observed due to a decrease in the responsiveness to flow stimulation in cilia-defective PKD (kidney-specific inactivation of *Kif3a*) models (55, 82), suggesting that proteins localized to the basal body of the cilium and normal ciliary structure are critical to regulate mTOR signaling in PKD.

A new ciliary pathway that promotes renal cyst formation was recently reported (56). According to many papers published so far, the presence of renal cilia seems to act as a suppressor for renal cyst growth, but recent studies have demonstrated that primary cilia devoid of polycystin proteins can activate renal cyst growth (56). To prove this idea, a combination of the cilia-defective PKD mouse model and the polycystin-defective mouse model was produced (56). Surprisingly, loss of renal cilia reduced renal cyst size following defects in polycystin proteins, suggesting a new pathway involving cilia-dependent cyst activating (CDCA) mechanisms inhibited by polycystin (56). However, a CDCA-specific pathway or regulator has not yet been identified, so further studies are needed.

In summary, normal primary cilia with polycystin proteins are critical to suppress rapid renal cyst growth by inhibiting the increase of cell proliferation, leading to the onset of PKD. In this chapter, MAPK, mTOR and CDCA pathways are discussed, but Hedgehog and Wnt pathways are also regulated in primary cilia and disrupted in PKD models. Therefore, identification of the role of cilia in PKD and role of polycystins in cilia are helpful to understand PKD pathogenesis and to identify new therapeutic targets for curing PKD.

Conclusion

The relationship between defects in primary cilia and PKD development has been elucidated, but the exact role of the primary cilia and related proteins in PKD remains to be identified. It was recently suggested that various pathogenic proteins observed in PKD models are localized to primary cilia. Also, it has been reported that proteins associated with primary ciliary assembly or with regulating ciliary function play a role in regulating the cell cycle in non-ciliated cells (83), which suggests that ciliary proteins may be essential

for regulating multiple signaling pathways in a cilia-dependent as well as cilia-independent manner. Therefore, elucidating the role of primary cilia or components related to primary cilia will provide new insights into the pathological mechanisms of ciliopathies involving PKD in addition to non-ciliopathies.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 16

Implications of Dysfunction of Mechanosensory Cilia in Polycystic Kidney Disease

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Abstract

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a multisystemic disorder characterized by numerous fluid-filled renal cysts that eventually destroy the kidney architecture and lead to end-stage kidney disease (ESKD). Although the formation of bilateral cystic kidneys is the hallmark of the disease, patients with ADPKD also suffer from extra-renal manifestations and cardiovascular complications. ADPKD is considered

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a ciliopathy disease due to defects in mechanosensory polycystins, localized to primary cilia, which have been recognized as mechanosensory organelles due to their involvement in ADPKD only within the past decade. Our recent studies focus on the fluid mechanosensory functions of primary cilia using cultured cells, animal models, and tissue from ADPKD patients. Growing evidence from these studies suggests that aberrant expression or localization of polycystins to cilia could promote high blood pressure due to the inability to synthesize nitric oxide in response to an increase in shear stress, and alteration of function of cilia could contribute to vascular and renal abnormalities in ADPKD. Our results have led us to propose that drugs targeting primary ciliary function could to be a novel therapeutic approach to slow the progression of pathogenesis in ADPKD. In this chapter, in order to explain the involvement of primary cilia in ADPKD, the structure of primary cilia and their mechanosensory function will be described and their contribution to diseases of the kidney and cardiovascular system will be discussed in regards to ADPKD.

Key words: Fluid flow; Mechanosensing; Polycystic kidney; Primary cilium; Shear stress

Introduction

Polycystic kidney disease (PKD) is the most widely inherited kidney disease and a leading cause of end stage kidney disease (ESKD) in both adults and children (1, 2). Affecting 1:400 to 1:1000 people, it is primarily characterized by renal cystogenesis; however, multisystemic complications are seen in both cystic and non-cystic phenotypes (3). PKD can be categorized into two types, autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). ADPKD has the highest occurrence rate, but clinical manifestations do not develop until 30 or 40 years of age. It is caused by a mutation in either *PKD1* or *PKD2* genes encoding for polycystin-1 (PC-1) or polycystin-2 (PC-2), respectively (4, 5). ARPKD is significantly less common (1 in 20,000 live births), predominantly affecting children (6). ARPKD is caused by a mutation in *PKHD1*, the gene encoding for fibrocystin/polyductin (FPC). Though the chief clinical manifestation is still bilateral cyst development, this mutation, in combination with others, may be the cause of the increased severity of the disease and the broad phenotypic variance (7). One of the key facets of PKD is ciliary dysfunction, which contributes to different cellular pathologies related to planar cell polarity, cellular differentiation, cell signaling mechanisms, and fluid sensing and transport (8). Ciliary alterations not only cause renal manifestations but also lead to extra-renal complications in the liver and cardiovascular system (9, 10).

Etiology

ADPKD is a completely penetrative heterogeneous disease associated with mutations at two loci, *PKD1* on chromosome 16 or *PKD2* on chromosome 4 (2, 11). Mutations in *PKD1* are responsible for about 85% of all PKD cases while mutations in *PKD2* only cause about 15% of the cases. The PC-1 and PC-2 proteins form a mechanosensory complex in the ciliary membrane that requires both proteins in order to function properly. Henceforth, either mutation will display identical phenotypes (12). PC-1 is a large transmembrane protein that functions as a mechanosensor and/or a chemosensor. PC-2, on the other hand, is a calcium channel that requires PC-1 to function properly (13).

A mutation in the *PKHD1* gene on chromosome 6 leads to ARPKD, a rare pediatric form of polycystic kidney disease. The *PKHD1* gene product, FPC, has also been found to localize to the cilia. It remains uncertain what the exact function of fibrocystin is; however, several studies show that FPC interacts with PC-2 but some suggest there may also be an interaction with PC-1 (6, 14, 15). Due to the wide genotypic variance in *PKHD1*, the clinical phenotypes also vary, even amongst family members (16). Renal cysts also characterize the clinical manifestations, and most patients will progress to ESKD. Unlike ADPKD, in which patients may or may not develop hepatic cysts, all patients with ARPKD will exhibit hepatic phenotypes, specifically a ductal plate malformation. This malformation in turn causes the biliary duct to dilate and macroscopic cysts to form (6).

Clinical manifestations

The formation of bilateral cystic kidneys is the hallmark of the disease. However, patients with PKD also suffer from extra-renal manifestations and cardiovascular complications.

Kidney

Due to cyst growth, the renal parenchyma is disturbed, altering normal kidney architecture and leading to variations in size (150 cm³ to >1500 cm³) as well as marked asymmetry between the kidney pairs (17, 18) (Figure 1). An increased expression of proto-oncogenes in the kidney leads to uncontrolled cellular proliferation of the cells lining the lumen, initiating cystogenesis. Glomerular filtrate accumulates within the cysts while still connected to the originating tubule. Once the cyst separates from the lumen, fluid accumulates through the secretion of transepithelial fluid, leading to continued cyst enlargement. In ADPKD patients, the cysts are located through the cortical and medullar regions of the kidney and are only produced by a small fraction

of nephrons. Generally, the fluid within the developed cysts is similar to urine in appearance, but there are cases in which the fluid is very dark with a paste-like consistency (17).

ARPKD typically presents prenatally and the kidney phenotype is characterized by nonobstructive cystogenesis occurring through symmetric dilatation as well as an elongation of the collecting duct by 10- 90%. In stark contrast to ADPKD, the kidneys in ARPKD maintain their integrity mainly due to the fact that the cysts are typically smaller and the malformation is primarily caused by the increase in the collecting duct size (19). Though parts of the cellular proliferation pathways are thought to be similar in both ADPKD and ARPKD, the distinct anatomical difference in cysts suggests a different secretion pathway. Currently, it is thought that cystogenesis in ARPKD patients is caused by a decrease in sodium absorption leading to altered fluid secretion (20, 21).

Hematuria is seen in more than 40% of ADPKD patients and usually resolves itself within a week. This diagnosis can be based on a variety of presentations such as hemorrhagic cysts, renal stones, urinary tract infections, or abdominal trauma. Hypertensive ADPKD patients are significantly more likely to experience hematuria than are normotensive ADPKD patients (10).

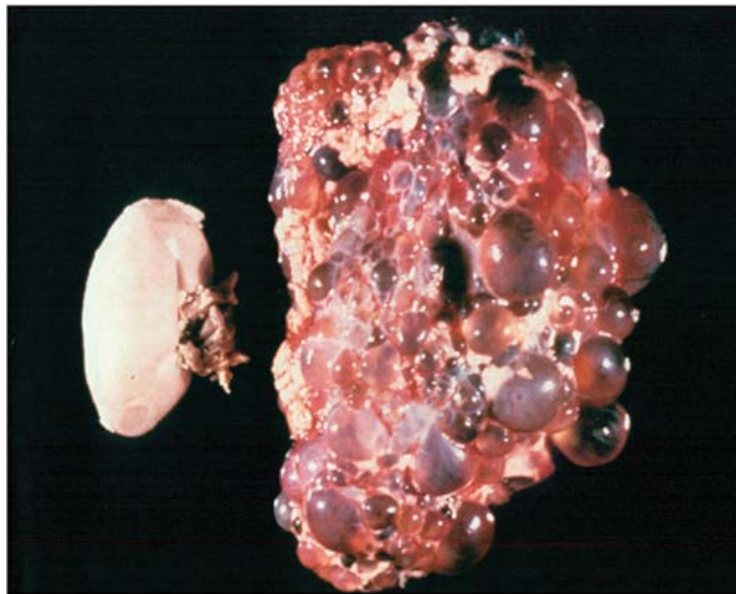


Figure 1. Cystic kidney. Comparison between a normal human kidney (left) and a cystic kidney (right). This image was adopted from (77) with permission.

Primary cilia and polycystic kidney disease

Nephrolithiasis occurs more frequently in ADPKD patients due to the presence of cysts that hinder the urinary collection process. However, metabolic alterations also play a role in stone development. ADPKD patients are more likely to have metabolic problems such as hypocitraturia or hyperuricemia, leading to renal stones with specific compositions (22).

Poor fluid management, such as nocturia, is also commonly seen in ADPKD patients. In the normal population, a loss of ability to concentrate urine is typically associated with old age; however, in the ADPKD population, it displays as an early symptom of the disease (17).

Liver

One of the more common extra-renal manifestations in ADPKD is the formation of hepatic cysts, which develop later than the renal cysts and for which incidence rates increase with age (5). The cysts develop due to a combination of a malformation in the ductal plate and dysfunctional primary cilia. The ductal plate is a single layer of hepatoblasts that surround the portal vein; through a sequence of growth and apoptosis, the hepatoblasts form a double layer that eventually becomes the bile duct (23). The cystic abnormality occurs when intralobular bile ductules remain disconnected (von Meyenburg complexes) and begin to dilate, due to the inability to undergo apoptosis (24, 25). Cilia in the liver stem from specific cells called cholangiocytes. The cilia have a mechanosensory function. Cilia bending in response to fluid flow help regulate intracellular levels of cAMP and calcium. Thus, deficient cilia result in altered levels of calcium and cAMP, causing increased proliferation of cholangiocytes. They also function to detect the osmolality and composition of bile. As such, the defective cilia are also unable to regulate the fluid flow, absorption, and secretion within the lumen (26).

Cardiovascular

Cardiovascular complications such as hypertension, aneurysm, left ventricular hypertrophy, and mitral valve prolapse are the leading cause of death for ADPKD patients (27). The overall cardiovascular abnormalities contribute significantly to morbidity and mortality in ADPKD patients (27, 28). Due to the focus on the extreme pathology of the renal system in ADPKD patients, the cardiovascular prevalence in ADPKD has unfortunately not been well studied.

Hypertension is the most common risk factor in ADPKD. It can be used as a diagnostic marker for ESKD simply due to its occurrence before the onset of renal dysfunction (29). The incidence of hypertension in ADPKD patients is usually one to two decades earlier than in the general population and differs between males and females. Increased blood pressure has been reported among children with ADPKD with an association with target organ damage, specifically if at least one of the parents is hypertensive (27, 30). Hypertensive ADPKD

patients often exhibit left ventricular hypertrophy (LVH), biventricular diastolic dysfunction and impaired coronary blood flow (31). However, the incidence of hypertension alone is not sufficient to explain these cardiovascular phenotypes, as a significant fraction of normotensive ADPKD patients exhibit some cardiovascular abnormalities. Thus, it is safe to assume that additional factors contribute to the pathogenesis of cardiovascular abnormalities. In addition, hypertension is associated with increased kidney volume and decreased kidney function in ADPKD. Evidence suggests the possible involvement of the renin angiotensin aldosterone system (RAAS) in this association, though the exact mechanism is unknown. Taken together, early diagnosis and treatment of hypertension will definitely lead to a decrease in the prevalence of target organ damage in ADPKD patients.

Intracranial aneurysms are significantly more common in ADPKD patients than in the general population and are responsible for 4-7% of the deaths in ADPKD patients. Furthermore, in ADPKD patients, intracranial aneurysmal development and rupture both occur at a younger age than in the general population. ADPKD-associated aneurysms are not limited to the cranial arteries, but have also been reported in the coronary arteries, abdominal aorta, renal artery, and splenic artery. With the expression of PC-1 and PC-2 in vascular smooth muscle cells, it stands to reason that these proteins have a potential role in the pathogenesis of aneurysms. Currently, clinical testing and diagnosis is routinely performed in ADPKD patients with a family history of aneurysms, since the incidence rate of aneurysm formation is double that of the ADPKD population with no known history. Nevertheless, patients who have no prior history of aneurysms, but have experienced a subarachnoid hemorrhage, are also screened (5).

LVH has been reported in roughly half of hypertensive ADPKD patients. In ADPKD patients, an increase in left ventricular mass index (LVMI) has been associated with poor renal prognosis as well as negative overall outcomes. Increased LVMI has also been reported in normotensive ADPKD patients with preserved renal function, which is suggestive of diastolic dysfunction, an abnormality seen in both normotensive and hypertensive ADPKD patients (31).

Cardiac valvular abnormalities have also been reported in ADPKD patients, with mitral valve prolapse being one of the more common malformations. Several studies have reported an approximate 25% incidence rate of mitral valve prolapse and a 30% incidence rate of mitral incompetence (27).

Screening, diagnosis and therapies

ADPKD is relatively easy to diagnose, given the characteristic renal cyst development. Familial history also aids in the diagnostic process and can be useful for a presymptomatic

diagnosis. An enlarged kidney or liver and cardiovascular issues such as hypertension or mitral valve prolapse in patients with a known family history of ADPKD is highly suggestive of a positive diagnosis. For those whose family history is unknown, bilateral renal enlargement and cysts, along with an absence of phenotypes suggesting other cystic diseases, leads to a more ostensive diagnosis requiring further tests. A diagnosis is confirmed through either imaging or genetic testing, regardless of familial history (32). Most commonly, an ultrasound is used to detect the classic symptoms such as bilateral cysts; however, imaging becomes unreliable under certain circumstances. Children suffering from early stages of ADPKD have smaller cysts that may escape sonographic detection; this is also frequently the case for patients suffering from the milder ADPKD type 2 (33). An age-dependent algorithm for ADPKD type 1 was developed for at-risk patients to assess a potential diagnosis based on the number of cysts an individual has at a certain age. This algorithm was later modified for more stringent parameters to aid in the diagnosis of ADPKD type 2 patients. For ADPKD type 1 patients, two unilateral or bilateral cysts between the ages of 15-29 years is a valid indication of a potential diagnosis, while for ADPKD type 2 patients aged 15-39 years, at least three unilateral or bilateral cysts is the parameter (33, 34). Computerized tomography (CT) scans and magnetic resonance imaging (MRI) are occasionally used in diagnosis, since their detection sensitivity is much greater than that of ultrasound; however, they are more expensive (35, 36). In addition to imaging techniques, genetic tests such as sequence analysis and duplication/deletions analysis are used to confirm PKD diagnosis. Sequence analysis detects small insertions/deletions, missense, and splice-site mutations that account for genetic variances from benign to pathogenic. Larger mutations such as duplications or deletions of a complete gene are not detected in this method but can be detected through duplication/deletion analysis (32).

Though there are no targeted ADPKD therapies that are clinically approved, the current treatment strategies focus on slowing cyst formation and treating the associated complications. Mammalian target of rapamycin (mTOR) inhibitors have been considered as potential therapeutic agents for ADPKD due to their ability to inhibit cellular proliferation and cyst growth. In a retrospective study, rapamycin proved to be more effective than cyclosporine in preventing kidney enlargement after renal transplants in ADPKD patients, which supported mTOR inhibitors as potential therapies. Unfortunately, upon clinical trials in ADPKD patients, mTOR inhibitors have shown disappointing results. An 18-month study of rapamycin therapy in ADPKD patients had no effect on the total kidney volume (TKV), but seemed to slow the decline of renal function (37). Worse yet, a two-year study with everolimus (a rapamycin analog) showed an increase in TKV and worsened renal function.

Somatostatin has also been studied as a therapeutic target for ADPKD and polycystic liver disease (PLD) due to its ability to decrease cAMP levels in tubular epithelial cells and

cholangiocytes. One study provided octreotide, a somatostatin analogue, to ADPKD and PLD patients over the course of one year. Compared to the control group, there was a significant decrease in changes in liver volume, but TKV remained stable. The patients in the octreotide group reported less pain and were able to increase their physical activity (1).

Currently, Tolvaptan therapy has the most promising results. Arginine vasopressin stimulates cAMP production in the distal nephron and collecting duct; Tolvaptan antagonizes vasopressin V2 receptor, thereby inhibiting cAMP production. Tolvaptan is well into the clinical trial process, with phase-2 results reporting that it is well tolerated in ADPKD patients. After a three-year study with the therapy, the rate of increasing TKV and renal function decline was significantly slowed; however, adverse effects caused 25% of the patients to discontinue the drug (38).

Unfortunately, the treatment options for those who progress to ESKD are limited to dialysis and renal transplantation (39). Patients with ESKD generally respond well to peritoneal dialysis, assuming there is enough intraperitoneal space within the kidneys to support the increased fluid volume. The lack of space due to kidney enlargement increases the risk of hernia development; therefore, it is often not the primary choice for treatment of ADPKD-induced ESKD (40). The primary course of treatment is renal transplant surgery, considering that the surgery risks for ADPKD patients are no greater than those for patients with ESKD from other diseases (41).

Primary cilia and polycystic kidney disease

ADPKD is a pathology associated with ciliary dysfunction, also known as ciliopathy. Ciliopathy is a general term used to collectively describe genetic disorders caused by mutations that affect the structure and function of the cilia or the basal bodies.

Ciliary structure

Non-motile primary cilia, as both chemosensory and mechanosensory organelles, act as an antenna on the apical surface of most cells to sense and transmit information from the extracellular matrix (ECM) to the cell interior. The primary cilium is a microtubule-based organelle that originates from the basal body of the mature centriole in quiescent cells (42). During cell division, the cilium is reabsorbed into the cell, allowing the centrioles to reform into the centrosome. As the cell re-enters the G₀ phase of cell division, the mature centriole migrates towards the cell surface to anchor just below the cell membrane, where it becomes the basal body (43). The basal body is a cylindrically-arranged group of nine triplet

microtubules (A, B, and C) responsible for both producing the ciliary skeleton and anchoring the organelle to the cell (44). The termination of the basal body is marked by the transition of the C microtubule into transition fibers that attach the basal body to the cell membrane. Microtubules A and B remain and continue to elongate through the process of intraflagellar transport (45), creating the concentric nine doublet microtubules of the cilium structure, known as the axoneme (46, 47). IFT is a process that uses the bidirectional movement of cargo proteins located between the axoneme and the ciliary membrane. Kinesin-2 motors are responsible for anterograde movement associated with cilia assembly, while dynein-2 motor proteins transport particles from the tip to the base of the cilium for recycling. The IFT system is essential for ciliogenesis as well as for the signaling cascades elicited through normal function of cilia (48). The ciliary membrane, distinct from the cell membrane, encases the microtubules of the axoneme. The ciliary necklace constitutes a series of proteins at the transition zone that separate the ciliary and cell membranes (Figure 2) (49, 50).

Ciliary function

Although primary cilia have been reported in the literature since 1968, it was only recently that cilia were considered to be more than vestigial organelles (51, 52). The normal length of primary cilia is between 5 to 10 μm , and cilia extend into the extracellular environment, making them ideal chemosensory and mechanosensory organelles (43). Studies have demonstrated the roles of primary cilia in various organs and structures, including but not limited to the kidneys (53), blood vessels (8), heart (54), liver (55), bone (56), retina (57), nose (58), and inner ear (59).

The mechanosensory function of primary cilia in the kidney is one of their most widely studied functions. Many receptors have been localized to renal primary cilia, thus implying a chemosensory function also involved in the complex signaling cascades for cell and tissue homeostasis (53). Cilia sense fluid flow, a function that is essential for proper kidney function (60); however, detection of fluid flow requires the functioning mechanosensory complex that forms between PC-1 and PC-2. Primary cilia are activated in response to shear stress. This in turn leads to the primary cilia bending and the opening of the PC-2 calcium channel, resulting in the influx of calcium ions and the increase in intracellular calcium concentration. Intracellular calcium acts as a second messenger for multiple signaling pathways in the cell (Figure 3). Following the cessation of the stimulus, PC-1 is cleaved and acts as a transcription activator in conjunction with several other pathways (61). It is therefore not surprising that a mutation in genes responsible for PC-1, PC-2 or fibrocystin would result in polycystic kidney disease. This further suggests that dysfunction of renal cilia in response to urine flow would result in polycystic kidney disease.

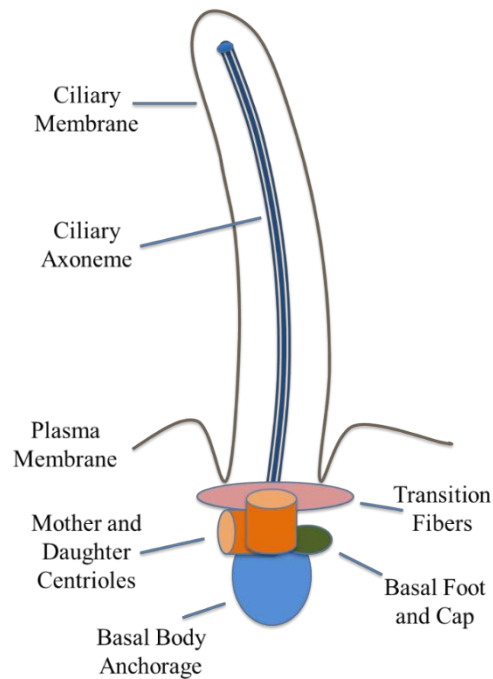


Figure 2. Illustration of major structures of primary cilia. The ciliary membrane, axoneme, and basal body constitute the basic structure of primary cilia. The basal body is composed primarily of the mother and daughter centrioles with transition fibers to assist in anchoring the basal body to the plasma membrane. The axoneme, which forms the ciliary skeleton, originates from the mother centriole. The ciliary membrane houses specific protein receptors and channels that enable proper cilia function.

Dysfunctional primary cilia can contribute to the pathogenesis of polycystic kidney disease through planar cell polarity (PCP), an organized arrangement of cells in a plane of tissue perpendicular to the apical-basal axis as a direction for the orientation of cell division (62- 64). One of the signaling pathways regulated by cilia activation is the Wnt (Wingless type mouse mammary tumor virus) signaling pathway, which can be subdivided into two types, canonical and non-canonical pathways. The canonical pathway stabilizes β -catenin, a transcription factor that helps regulate gene expression. The canonical pathway also helps regulate cellular proliferation, differentiation and fate. The non-canonical pathway (PCP), in contrast, degrades β -catenin but also influences cytoskeletal organization and morphogenesis. The receptors responsible for determining which pathway is activated have been localized to primary cilia (43, 65). Using different mouse models of cystic kidney disease, defects in any ciliary protein lead to abnormal orientation during cell division. It is therefore thought that inactivation of ciliary proteins would result in abnormal PCP, which in turn triggers an increase in tubular diameter in the kidney (Figure 4). The net result is cystic formation in the kidney.

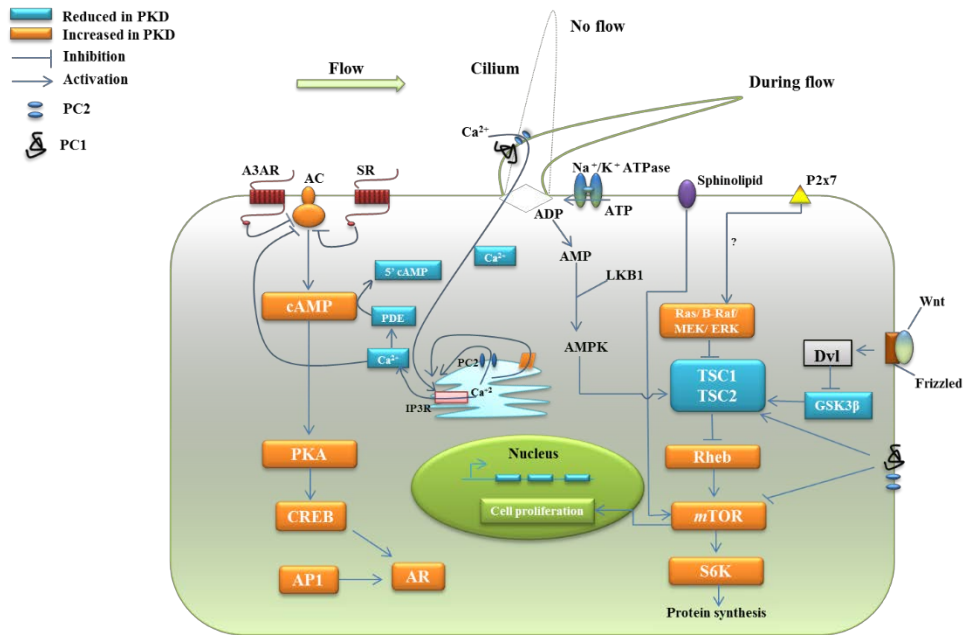


Figure 3. Cilia are mechanosensory organelles. Cilia are sensory organelles that sense fluid-shear stress on the apical membrane of the cells. Fluid flow that produces enough drag-force on the cells will bend sensory cilia. The diagram illustrates the mechanism that polycystin-1 (PC1), polycystin-2 (PC2), signaling proteins, molecules and other receptors exert on signaling pathways, leading to cyst formation. The blue box indicates the reduced molecules and signaling proteins in ADPKD. The orange box indicates the increased signaling proteins in ADPKD, which are thought to be responsible for an increase in cell proliferation, including cAMP, Ras/Raf/ERK, adenylate cyclase (AC), and mTOR activity. In addition, EGFR activation is also enhanced by amphiregulin (AR), which is abnormally expressed in cystic cells through cAMP, CREB and AP1 signaling (not shown). The sphonigolipid, Na⁺/K⁺ ATPase, Wnt and P2x7 purinergic receptors are also involved in the regulation of mTOR and TSC1/TSC2 complex activity. Other receptors that are involved in ADPKD include adenosine receptor-3A (A3AR) and somatostatin receptor (10), which regulate the activity of AC. This illustration was adopted from (53).

Another pathway that is linked to primary cilia function is Hedgehog (Hh) signaling which is essential for proper cell proliferation, differentiation, and general tissue homeostasis. The Patched 1 receptor (PTCH1), responsible for binding sonic hedgehog ligand, has been localized to primary cilia. Once the receptor is activated, it diminishes from the cilia and initiates a sequence of complex processes enabling normal renal development and homeostasis. Hh also regulates gene expression through suppression and activation of several transcription factors that have been localized to primary cilia. Several studies

provided evidence for the implication of Hh in renal cyst formation. Evidence suggests that renal cystogenesis is associated with enhanced Hh activity and that Sonic Hh mutant mice are characterized by abnormal kidney development (66, 67).

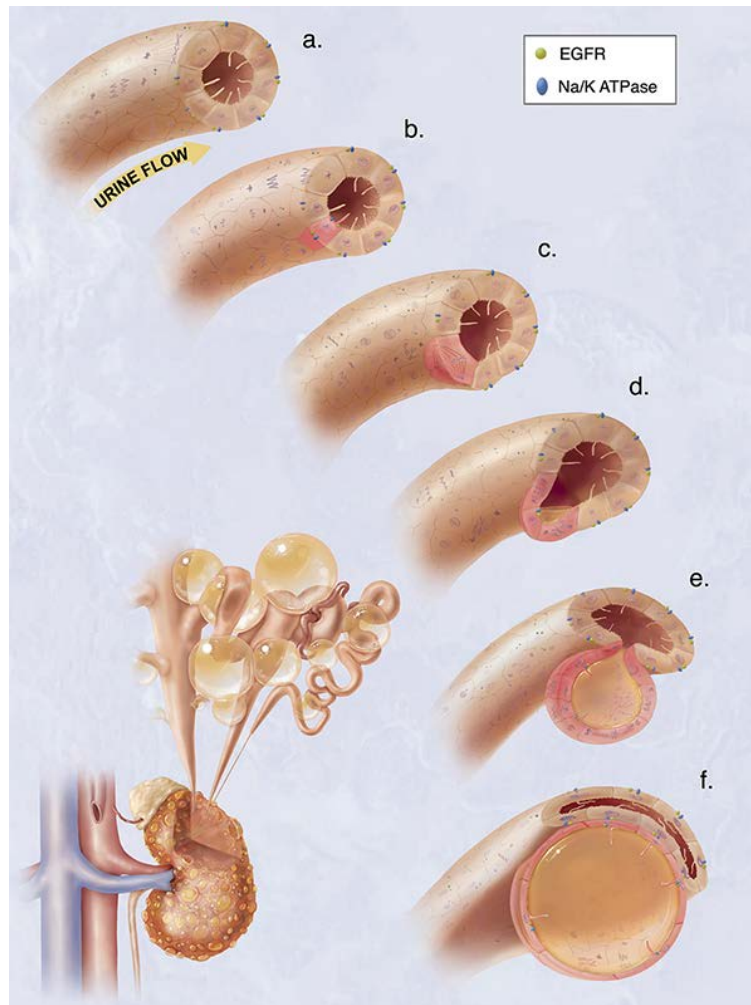


Figure 4. Cyst formation. a. Normal kidney tubule structure; urine can easily pass. The cilia are able to sense the fluid flow and respond accordingly. b. Mutations in the cilia due to genetic disorders such as ADPKD prevent the cilia from functioning properly and thereby hinder response pathways. c. Due to the mutation, planar cell polarity is lost, inhibiting the cells from dividing in the proper orientation. d. The tubular diameter increases and begins to fill with fluid. e. The cyst begins to bud and separate from the nephron. f. The cyst continues to fill with fluid through absorption and secretion processes, causing continued enlargement and reducing the diameter of the nephron. Figure is reproduced under license from the original publisher (78).

Although the molecular mechanisms by which Hh signaling might contribute to kidney cyst formation are still under investigation, numerous reports suggest that decreased Hh activity assists renal epithelial cells in sustaining differentiation (68). Other studies suggest a link between calcium levels and renal cystogenesis through Hh signaling (67, 69). It has been demonstrated that Hh activity can modulate calcium signaling at both the intracellular and the primary ciliary levels.

Notch signaling uses surface receptors to link the fate of one cell to its neighbor, thereby influencing differentiation, proliferation, and apoptosis. Though notch is known to be essential for proper kidney development, it has not been widely studied in PKD patients. Ligand binding leads to the activation of notch receptors and results in the cleavage of their intracellular domains. These domains translocate to the nucleus and activate target gene expression through a series of complex protein binding interactions. Only recently, notch signaling has been shown to be regulated by primary cilia. Notch3 receptor was localized to the primary cilia of skin cells and *kif3a* knockout, a mutation that prevents cilia formation, caused the ablation of the nuclear localization of notch intracellular domains. In addition, *ift88* knockdown led to the differentiation failure in mouse keratinocytes and decreased notch activity.

The presence and the function of primary cilia in vascular endothelial cells lining the inner wall of blood vessels have been previously demonstrated (8). Primary endothelial cilia function as fluid shear stress mechanosensors to sense changes in blood flow and pressure and convert these mechanical changes into biochemical signals that regulate smooth muscle tone and blood pressure. The presence of endothelial cell primary cilia has been demonstrated in mouse aortic endothelial cells *in vitro*, in isolated mouse arteries *ex vivo*, and in mouse models as well as in blood vessels from human patients *in vivo* (5, 7). Primary endothelial cilia can detect changes in blood pressure or shear stress through the mechanosensory complex PC-1/PC-2. This in turn leads to the influx of calcium ions through the calcium channel, PC-2. As a result, an increase in intracellular calcium concentration triggers a series of signaling cascades that result in the activation of endothelial nitric oxide synthase (eNOS) and the release of NO gas, which diffuses to the surrounding vascular smooth muscle cells, causing vasodilation. Therefore, the inability of ciliary polycystins to sense fluid shear stress due to mutations might contribute in part to hypertension in ADPKD patients. In addition, our recent studies have provided evidence for the involvement of primary endothelial cilia in signaling pathways that regulate vascular architecture and have shown that cilia dysfunction can contribute to aneurysm formation through downregulation of survivin (70). All in all, this led us to propose primary endothelial cilia as a potential therapeutic target for vascular disease (Figure 5).

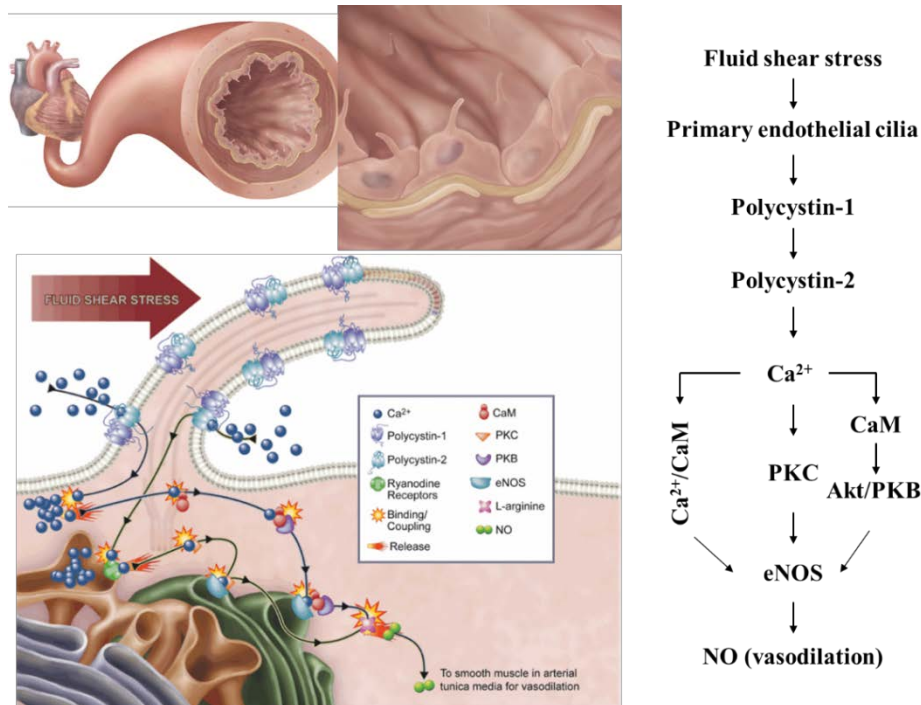


Figure 5. The role of mechanosensory cilia and nitric oxide production in ADPKD. The illustration depicts the activation of nitric oxide (NO) during fluid shear stress. The biochemical production and release of NO is dependent on the function of endothelial cilia in the vasculature. In ADPKD, dysfunctional cilia are not able to sense blood flow mechanically, and NO is not produced, resulting in increased blood pressure. The bending of cilia by fluid-shear stress activates the mechanosensory polycystins complex and initiates biochemical synthesis and the release of NO. This biochemical cascade involves extracellular calcium influx, followed by the activation of various calcium-dependent proteins, including calmodulin (79), protein kinase C (PKC) and Akt/PKB). Figure is modified from (80).

Vascular smooth muscle cells (VSMC) are constantly under stress due to blood flow, arterial wall stretching, and constriction. This dynamic environment requires transmission of mechanical and chemical perturbations to the VSMC through the ECM (71). VSMCs are ciliated, though they do not come into direct contact with fluid flow unless there is an injury to the arterial cell wall. The VSMC cilia have a mechanosensory function and are specifically oriented along the arterial axis at a 60° angle (72, 73). It is thought that the angle assists in enhancing the signal transduction by increasing the contact area with the ECM. These cilia are implicated in cellular migration and respond to fluid flow, suggesting a role in intracellular calcium levels (72). Upon injury to the arterial wall, a study found that deciliated VSMC showed a reduced cell migration and thus a reduction in healing efficacy (71). The

mechanical force placed on cilia cause them to bend, initiating a calcium influx through the PC-2 channel that is proportional to the degree of cilia bending.

Cellular signaling pathways in the heart are regulated by cardiac primary cilia that localize in cardiomyocytes. In the developing heart, cardiac primary cilia have been implicated in signaling cascades associated with morphogenesis, differentiation, and maturation (54). For example, primary cilia may be involved in the highly conserved left-right (LR) asymmetry that manifests in the heart, as well as throughout the body. A type of motile cilia called nodal cilia primarily dictates LR asymmetry by moving in a rotatory motion, pushing embryonic fluid (nodal flow) toward the left. It is suggested that the nodal flow activates the PC-2 calcium channel of non-motile primary cilia (74). As the heart continues to develop, it is also suggested that the primary cilia begin to reabsorb from the endothelium of the endocardial cushion in response to shear stress. Shear stress is essential for proper cardiomyocyte proliferation and development of the conduction system. Mutations in cilia structure and/or polycystins have led to impaired fluid flow sensing, causing aberrations in cardiac maturation (54).

As mentioned earlier, cholangiocyte cilia are preferentially oriented towards the bile duct lumen to enable proper monitoring of bile flow and changes in bile composition. These changes are in turn associated with signaling cascades responsible for cholangiocyte proliferation and secretion (55). The multisensory functions and implications of cholangiocyte cilia are being investigated fervently. The polycystins are mechanosensory molecules crucial for calcium signaling associated with primary cilia; however, cholangiocyte cilia also host calcium-inhibitable adenylyl cyclase 6 (AC6), an axoneme-localized protein involved in cAMP signaling (75).

In summary, it has been well documented that cilia bending in response to fluid shear stress, trigger intracellular calcium signaling. However; recent studies from Nauli's group have shown that calcium signaling triggered by fluid shear stress is initiated rather in the cilioplasm and can be spatially and temporally differentiated from the subsequent intracellular cytosolic calcium. The authors suggest that the flow-induced calcium signaling is mainly dependent on the ciliary mechanosensor, PC-2. This is mainly supported by data demonstrating that ciliary activation by the dopamine receptor-5 (DR-5)-specific agonist induces calcium signaling only in the cilioplasm while thrombin treatment induced cytosolic signaling through the IP₃ receptors. This led the authors to conclude that cilium-dependent signaling can either be contained within the cilioplasm or spread to the cytoplasm. Henceforth, proposing cilia as a calcium signaling compartment in addition to its function as a sensory organelle (76).

In contrast to the above hypothesis, the Clapham group has recently proposed cilia as unique calcium compartments regulated by a heteromeric TRP channel, PKD1-L1/PKD2-L1.

According to their studies, ciliary calcium concentrations fluctuates in response to external stimuli, such as rupturing the ciliary membrane at the tip of the cilia with a laser pulse, without substantial effect on cytosolic calcium levels (69). While both studies concluded that cilia can function as a calcium compartment, it remains difficult to compare the difference in the overarching conclusions regarding changes in ciliary and subsequent cytosolic calcium levels simply due to the difference in the approaches employed to study ciliary calcium signaling.

Conclusion

ADPKD is a ciliopathy disease characterized by the formation of kidney cysts, vascular aneurysms and hypertension. Surprisingly, these complications in the kidney tubules and blood vessels are associated with one another. Abnormal renal epithelial ciliary function in sensing urine flow can lead to kidney cyst formation. The inability of vascular endothelial cilia to sense blood flow can induce hypertension and vascular aneurysms. However, the formation of renal cysts or vascular aneurysms may or may not be dependent on the incidence of hypertension. Primary cilia, as newly-discovered mechanosensory and chemosensory organelles, have important roles in disease and development. Defects in primary cilia can trigger a wide range of pathologies in the kidney, heart, vasculatures and many other organs. The importance of sensory cilia in other organ systems is yet to be discovered, and many more cilia-related diseases are still to be identified. There is no doubt that the physiological roles of primary cilia will continue to be debated in years to come.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 17

The Liver and Polycystic Kidney Disease

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Abstract

The hereditary forms of polycystic kidney disease (PKD) include a wide range of heterogeneous diseases of great clinical importance, of which autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD) are the main forms. ADPKD is a multifactorial disorder characterized by bilateral renal cysts and usually affects adult patients. Liver cysts are the most common extrarenal manifestations of ADPKD and are often incidental findings and clinically insignificant. In contrast, ARPKD is a severe, typically early-onset form of renal cystic disease. ARPKD patients may present with

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clinically-significant congenital hepatic fibrosis, with portal hypertension, requiring close monitoring, surgical shunting procedures, and kidney and/or liver transplantation. ARPKD is also related to Caroli's disease, a rare autosomal recessive congenital syndrome characterized by multiple saccular dilatations of intrahepatic bile ducts, with predisposition to gallstones, cholangitis and renal cysts. Simple hepatic cysts can also arise from excessive proliferation and dilatation of the bile ducts and peribiliary glands, which are rare in children but their frequency increases with age. The cystic liver epithelial cells have specific receptors, cytokines and growth factors that stimulate and promote cell proliferation and cyst formation. In general, hepatocellular function remains relatively preserved in this group of liver diseases, but may result in complications due to mass effects. The pathogenic sequence and genetic profile of PKD-associated liver cyst formation and progression is under extensive investigation. Therapeutic strategies to prevent and retard renal and liver cyst growth should be available in the near future.

Keywords: ADPLD; Caroli's disease; Hepatic cystic dilatations; Hepatorenal fibrocystic disease; Polycystic liver disease

Introduction

Adult liver cystic lesions are classified as hereditary or developmental, neoplastic, inflammatory, or mixed lesions. The hereditary forms of polycystic liver disease (PLD) are associated with autosomal dominant polycystic kidney disease (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD), or occur as a distinct genetic disease in the absence of renal cysts (1-3).

ADPKD is a multifactorial disorder characterized by the formation and growth of multiple fluid-filled renal cysts that progress over decades with attendant inflammation and fibrosis. The major extrarenal manifestation of ADPKD is PLD, which does not affect liver function, but represents a heterogeneous set of structural changes of the biliary tree development and causes symptoms related to mass effects when significant liver enlargement occurs (3-5). During fetal development, defects in genetic mechanisms and signaling pathways cause disruption in the biliary tree, leading to formation of cystic structures, which typically remain asymptomatic until adulthood, when they start growing under hormonal action and may become symptomatic (6). Often these cysts are incidental findings, and clinically insignificant. They are rare in children but their frequency increases with age in women, especially under hormonal influences such as pregnancies or estrogen replacement therapies (7). The hepatic cyst epithelial cells have estrogen receptors, growth factor similar to insulin-like growth factor-1 (IGF-1), other growth hormones and cytokines that when stimulated promote proliferation and cystic growth (8,9).

In contrast, ARPKD is considered the severe form of renal cystic disease, frequently with multisystemic manifestations, including congenital hepatic fibrosis and portal hypertension, and requiring close monitoring, surgical shunting procedures, and kidney or liver transplantation (10). In addition, ARPKD can also be related to Caroli's disease, a rare autosomal recessive congenital syndrome characterized by multiple saccular dilatations of the intrahepatic bile ducts with predisposition to gallstones, cholangitis and renal cysts (11).

In PKD-associated PLD, specific mutations are identified in biliary epithelial cells that produce increased differentiation, proliferation and secretion, which results in the formation of cysts. However, in isolated PLD, hepatic cysts arise from excessive proliferation and dilatation of the bile ducts and peribiliary glands. Without an association with PKD, PLD produces larger cysts but has fewer complications when compared to the form associated with PKD, termed Autosomal Dominant Polycystic Kidney and Liver Disease. The frequency of PLD increases with age and may be underestimated by tomography and ultrasonography (12-15). In this chapter we will discuss PLD as part of ADPKD, ARPKD and as a distinct genetic disease in the absence of renal cysts.

Autosomal dominant polycystic kidney disease

ADPKD is one of the most common monogenic diseases characterized by the progressive development of renal and extrarenal cysts, with important variability in clinical expression (12,14,15). It has worldwide prevalence and affects 0.2% of the general population, or every 400 to 1,000 births (15), while isolated PLD is less prevalent than 0.01% (7). ADPKD is genetically heterogeneous with two identified genes: PKD1, which is located on chromosome 16p; and PKD2, on chromosome 4q21. These genes encode proteins called polycystin-1 (PC1) and polycystin-2 (PC2), respectively. Mutations of PKD1 represent 86% of cases, while mutations of PKD2 represent the rest of 14% (13,16,17), which trigger the formation of renal and hepatic cysts (Table 1). Despite genetic mutation, other factors are also involved in cystogenesis, such as age, female sex, pregnancy and oral contraceptive use (9).

PLD, as the most common extrarenal manifestations of ADPKD (12,15), is characterized by multiple biliary cystic lesions localized in over 50% of the hepatic parenchyma. Cystic size can range from 20 to 30 cm to small microscopic nodules. Patients with ADPKD have associated PLD in 75% -90% of cases (17,18). In ADPKD, hepatic cysts develop later than the renal cysts. The hepatic cysts are often incidental findings and clinically insignificant, often presented for the first time in the fourth decade of life and continuing to grow gradually with age in number and size. They are infrequent before age 20, with an

estimated prevalence of 20% in the third decade to 70% in the seventh decade of life (19,20). Both sexes are affected; however, women have a higher prevalence. Exposure to estrogen during pregnancy, use of oral contraceptive pills or estrogen replacement therapy seems to accelerate its progression (7,20). In women, under hormonal influence, the cysts can grow quickly, and when they reach high volume, the cysts can cause liver parenchyma atrophy (21).

In ADPKD with extrarenal manifestations of PLD, the liver cysts arise from the expansion of the bile microhamartomas and peribiliary glands, resulting from bile ducts epithelium overgrowth in the intralobular portion (14,17,22,23).

Autosomal recessive polycystic kidney disease

ARPKD is rare and typically of childhood onset. It occurs in 1: 6.000 to 1: 50,000 live births (29-32). ARPKD is caused by mutations in a single gene, polycystic kidney and hepatic disease 1 (PKHD1) gene, encoding a protein called fibrocystin/polyductin (29,30-34). Once the mutations of fibrocystin/polyductin occur, the structure of tubular epithelial cells lead to polarity disorders and emergence of cysts (33). In ARPKD, there is a genotype-phenotype correlation, in that the presence of two completely inactivating PKHD1 mutations results in a more severe clinical outcome associated with perinatal mortality (29,30-32). Patients with at least one hypomorphic missense mutation have a more juvenile presentation, suggesting that a subset of missense changes result in reduced rather than absent function of the PKHD1 gene product (30,31,35). Fibrocystin localizes in the cortical and medullary collecting ducts and thick ascending limbs of the loop of Henle in the kidney, but is also expressed in the bilio-pancreatic tracts, and in salivary ductal epithelia (33). Fibrocystin regulates planar cell polarity, and the complete loss of this protein leads to loss of oriented cell division (34).

Table 1. Comparison between polycystic kidney disease associated with hepatic cysts and isolated hepatic polycystic disease (12-28)

Clinical form	Polycystic kidney disease associated with hepatic cysts	Isolated hepatic polycystic disease
Prevalence	0-20%	<0.01%
Type of inheritance	Autosomal Dominant	Autosomal Dominant
Mutated genes	PKD1; PKD2	Prkcsb; Sec63
Encoded proteins	Polycystin 1; Polycystin 2	Hepato Cystin; Sec 63 protein
Chromosomal locus	16p; 4q21	19p; 6q21

ARPKD is characterized by non-obstructive fusiform dilatation of the renal collecting ducts and malformations of the biliary tract, with ectasia of the bile ducts and periportal fibrosis (29). It can manifest in neonates with exaggerated kidney growth, intrauterine renal failure and pulmonary hypoplasia, or may present later with renal failure accompanied by portal and systemic hypertension (30). ARPKD is associated with a high level of morbidity and mortality in affected individuals who require close monitoring, surgical shunting procedures, and kidney and/or liver transplantation (29,32).

ARPKD is also related to the Caroli's disease, which is a rare congenital syndrome with the same pattern of inheritance (autosomal recessive) characterized by multiple saccular dilatations of the intrahepatic bile ducts, and with a predisposition to the formation of gallstones, to cholangitis and cystic kidney (11). It is common in childhood and the second decade of life and may be associated not only with different degrees of renal cysts, but also renal tubular ectasia, nephrocalcinosis, and interstitial fibrosis and renal failure (29-31).

Autosomal dominant polycystic liver disease

PLD is an inherited condition characterized by the presence of multiple scattered cysts of biliary origin throughout the liver parenchyma. PLD occurs not only as an extra-renal manifestation of ADPKD (MIM173900 and MIM173910), but also in patients with autosomal dominant PLD (ADPLD; MIM174050), an entity that is genetically distinct from ADPKD and not typically associated with renal cysts (30,35).

PLD is classified according to the number, size and the amount of remaining liver parenchyma (7):

- Type I - unlimited number of large cysts (greater than 10 cm);
- Type II - diffuse involvement of the hepatic parenchyma by multiple medium sized cysts with large remaining areas of hepatic parenchyma without cysts;
- Type III - diffuse and massive involvement of the hepatic parenchyma by small and medium cysts and only a few areas of normal liver parenchyma between the cysts, the most severe form of the disease.

The genes involved in ADPLD are *Prkcsb* and *Sec63* and encode hepatocystin and Sec63p, respectively (Table 1). Unlike other cystoproteins, hepatocystin and Sec63p are not ciliary proteins; they are components of the molecular machinery involved in the translocation, folding and quality control of newly synthesized glycoproteins in the endoplasmic reticulum (35-40). Most mutations are truncating and probably conduct to a complete loss

of the corresponding proteins and a defective processing of key regulators of biliary cell growth. The finding that PLD is caused by proteins involved in oligosaccharide processing was unexpected and implicates a new avenue for research into neocystogenesis, and might ultimately result in the identification of novel therapeutic drugs (39-41).

The gene products of *Prkcs* and *Esa63*, glucosidase II β and Sec63p, are located in the endoplasmic reticulum and they are responsible for quality control machinery through which 30% of proteins encoded by the human genome pass, yet heterozygous mutations in these genes manifest only with bile duct cysts indistinguishable from the liver phenotype in ADPKD. Mutations in the *Prkcs* and the *Sec63* genes solely determine hepatic cyst formation (40-42).

Pathogenesis of hepatic cysts

In the human embryo, the first sign of the bile ducts and the liver is the hepatic diverticulum, also known as the liver bud. For up to 8 weeks of gestation, the extrahepatic biliary tree develops through lengthening of the caudal part of the hepatic diverticulum. This structure is patent from the beginning and as it is, remains in continuity with the developing liver at all stages. The hepatic duct (*ductus hepaticus*) develops from the cranial part (*pars hepatica*) of the hepatic diverticulum. The distal portions of the right and left hepatic ducts develop from the extrahepatic ducts and are clearly defined tubular structures by 12 weeks of gestation. The proximal portions of the main hilar ducts derive from the first intrahepatic ductal plates. The extrahepatic bile ducts and the developing intrahepatic biliary tree maintain luminal continuity from the very start of organogenesis throughout further development. The normal development of intrahepatic bile ducts requires finely timed and precisely tuned epithelial-mesenchymal interactions, which proceed from the hilum of the liver toward its periphery along the branches of the developing portal vein. Lack of remodeling of the ductal plate results in the persistence of an excess of embryonic bile duct structures remaining in their primitive ductal plate configuration. This abnormality has been termed the ductal plate malformation (36-39).

Hepatoblast differentiation into a tubular biliary phenotype is stimulated by growth factors and signaling pathways, such as Notch, transforming growth factor- β (TGF- β) and Wnt. This transformation and cellular remodeling are completed after the 30th week of pregnancy. Intrahepatic ducts and extrahepatic ducts then merge and share the hepatic hilum. During the first year of life, the biliary system continues its development (37,38). Processes involved in hepatic cystogenesis include ductal plate malformation with concomitant

abnormal fluid secretion, altered cell-matrix interaction and cholangiocyte hyperproliferation. The ductal plate malformation is a developmental portobiliary system abnormality and the basis of the biliary liver disease that manifests with congenital hepatic fibrosis, Caroli's syndrome, and PLD (28,45-46). In spite of that, hepatocellular function remains relatively preserved in this group of liver diseases associated to ductal plate malformation (28).

Based on experimental models of bile dysmorphogenesis, a new classification for the defects of the ductal plate was recently proposed: 1) dedifferentiation of abnormal hepatoblasts; 2) failure in bile duct maturation; and 3) ductal expansion disturbance, (18). Bile duct formation requires a network of epithelial and mesenchymal interactions, the presence of growth factors and transcription to direct and guide the migration, adhesion and differentiation of cholangiocytes (4,36,37).

The genetic connection between ADPKD and ADPLD

ADPLD is associated with mutations in the PKD1 and PKD2 genes. Carriers of mutations in the PKD1 gene have more renal complications compared to patients with PKD2 mutations (16-18,30). Recently, it has been shown that glucosidase II α and Sec63p are required in mice for adequate expression of a functional complex of the polycystic kidney disease gene products, PC1 and PC2. The authors found that PC1 is the rate-limiting component of this complex and that there is a dose-response relationship between cystic dilation and levels of functional PC1 following mutation of *PrkcsH* or *Sec63*. Reduced expression of PC1 also sensitizes the kidney to cyst formation resulting from mutations in *Pkhd1*. Proteasome inhibition increases steady-state levels of PC1 in cells lacking glucosidase II β and reduces cyst growth in orthologous mouse models of human ADPLD (40,41,43).

In addition, Cnossen *et al.* (44) identified that the low density Lipoprotein Receptor-related Protein 5 (LRP5) gene is the third locus associated with isolated PLD. It has been postulated that LRP5 variants may render ADPKD patients more susceptible to the development of polycystic liver. Cnossen *et al.* (44) have demonstrated that this gene may also have a role in unlinked and sporadic ADPKD patients. The authors have identified a total of four different LRP5 variants that were predicted to be pathogenic by *in silico* tools. One ADPKD patient has a positive family history for ADPKD and variant LRP5 c.1680G>T; p.(Trp560Cys) segregated with the disease. Although two PKD1 variants probably affecting protein function were also identified, luciferase activity assays presented for three LRP5 variants significantly decreased signal activation of canonical Wnt signaling. This

study contributes to the genetic spectrum of ADPKD, especially by the study of canonical Wnt signaling pathway that provides new insights for its pathophysiology (44). Experimental models have shown that other genes may also be associated with renal cystogenesis, for example, HNF1 β mutations may affect the progression and outcome of the renal cyst formation as well as ADPLD (41).

Clinical symptoms

Typically, PLD is asymptomatic, but the symptoms become more frequent with age, and so increase as a result of increased life expectancy, especially in patients with ADPKD due to dialysis and transplantation. Hepatic cysts are more prevalent and hepatic cyst volume is larger in women than in men, and multiple bile cystic lesions vary from 20 to 30 cm to small microscopic nodules (41,42). The clinical course of PLD is relatively benign compared with ADPKD. The symptoms may result from mass effects or complications related to the cysts. The symptoms that are typically caused by mass effects are hepatomegaly and portal hypertension, ascites, jaundice, hemorrhage, dyspnea, early satiety and weight loss, gastro-oesophageal reflux and pain in the lower back region (42-45). Symptomatic cyst complications include cyst hemorrhage, infection, and rarely torsion, or rupture (46). Other complications of mass effect are vena cava compression and lower portal vein and bile duct compression that presents itself as obstructive jaundice (12,41,47,48).

Management

PLD patients had significantly lower quality of life compared to general population. The primary outcome measurement of PLD management is to reduce liver volume and relieve associated symptoms. Higher liver volumes were associated with a lower quality of life. Abdominal pain and dyspnea had a significant impact on this physical dimension. Supportive management with analgesics is the first-line treatment in patients with acute or chronic abdominal pain and tenderness (15,49-54).

The primary aim of PLD therapy is to reduce symptoms by curtailing hepatic cyst development. The treatment of choice is driven by individual complaints. Therapeutic interventions are not warranted in asymptomatic patients. Conservative treatment is recommended for most patients with PLD. For symptomatic patients, therapy should be directed to the prevalent symptoms. Recent advances in ADPKD pathophysiology have stimulated research for new therapeutic strategies. The primary aim is to interrupt cyst growth to allow abdominal decompression and ameliorate related symptoms. The target of

such drugs are abnormal cellular signaling cascades, that lead to deregulated proliferation, cell dedifferentiation, apoptosis and fluid secretion (1, 7,15, 17, 53-58).

Because of the proliferative effect of estrogen on hepatic cysts, oral contraceptives containing estrogen and menopausal estrogen therapy should be administered at the lowest effective dose, or avoided in patients with ADPKD. The first advice to females with PLD and ADPKD with a history of multiple pregnancies and prolonged exogenous estrogen exposure is to stop oral contraceptives. Although not formally investigated, the use other (non-systemic) contraceptives such as an intra-uterine device may be an acceptable alternative (53).

In recent years, several randomized clinical trials have been performed to study quality of life and the effects of diverse drugs on the growth of renal and hepatic cysts (54-59). Drugs that have been tested in randomized clinical trials include the mammalian target of rapamycin (mTOR) inhibitors, sirolimus and everolimus (60), somatostatin analogues such as octreotide, lanreotide, pasireotide (61,62), and most recently, the vasopressin V2 receptor antagonist, tolvaptan (63). Additional drugs are being tested, which include among others, the Src-ABL tyrosine kinase inhibitor, bosutinib, triptolide, histone deacetylase (HdaC6), Cdc25A phosphatase, miRNAs and metalloproteinases that attenuate growth of hepatic cysts (64). Many of these targets have been evaluated in pre-clinical trials, suggesting their value as potential new therapies. Additional therapeutic strategies to retard cyst growth aim at blood pressure control via inhibition of the renin-angiotensin aldosterone system and the sympathetic nervous system. Invasive procedures are required in a selective patient group with advanced PLD, ADPKD or liver failure (59,63,65). The different invasive approaches with possible beneficial outcomes include cyst aspiration and sclerosis, open or laparoscopic fenestration, liver resection with fenestration, and liver transplantation (1,7,15,17, 21,56,66,67-71).

Conclusions

PLD can either co-exist with ADPKD and ARPKD or occur alone as ADPLD. ADPKD and ARPKD as well as ADPLD are a group of genetic disorders initiated by mutations in several related genes, which results in the changes in cell signaling pathways to regulate cyst initiation and progression. The gene products of ADPLD may be required in mice for adequate expression of a functional complex of the ADPKD gene products, PC1 and PC2. The progression of the disease occurs throughout the patient's life with possible deterioration of renal and liver function. The main risk factors for growth of liver cysts are female sex, exogenous oestrogen use and multiple pregnancies. Individuals diagnosed

with isolated PLD or PKD associated PLD should be monitored for signs and symptoms. The main diagnostic methods of hepatic involvement are ultrasound, tomography, magnetic resonance imaging, and laparoscopy as an alternative. In selected cases, study of genetic mutations may be required.

PLD is usually benign, but can cause debilitating abdominal symptoms in some patients. In spite of that, hepatocellular function remains relatively normal, but major morbidity associated with hepatic fibrosis is portal hypertension, often leading to esophageal varices and hypersplenism. Although PLD is not typically associated with portal hypertension, but it may result in complications due to mass effects (vena cava compression, obstructive jaundice). Current radiological and surgical therapies for symptomatic patients include aspiration-sclerotherapy, fenestration, segmental hepatic resection and liver transplantation. Medical therapies that interact with regulatory mechanisms controlling expansion and growth of liver cysts are under investigation.

Conflict of Interest

The authors declare that they have no conflict of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 18

Seminal Vesicles in Autosomal Dominant Polycystic Kidney Disease

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Abstract

Extra-renal manifestations of autosomal dominant polycystic kidney disease (ADPKD) have been known to involve male reproductive organs, including cysts in testis, epididymis, seminal vesicles, and prostate. The reported prevalence of seminal vesicle cysts in patients with ADPKD varies widely, from 6% by computed tomography (CT) to 21%–60% by transrectal ultrasonography. However, seminal vesicles in ADPKD that are dilated, with a diameter greater than 10 mm by magnetic resonance imaging (MRI), are

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“megavesicles”. This is a separate entity from seminal vesicle cysts and has a prevalence of 23% in ADPKD patients, but is not known to occur in patients without ADPKD. The basis of these cystic changes and megavesicles has not been established, but may be explained by an imbalance between cell growth/proliferation inhibitors and stimulators analogous to mechanisms in renal tubular epithelial cells, hepatic ducts, and in the vasculature. Male infertility has been associated with ADPKD, although a causal role of seminal tract abnormalities has not been established. In this chapter, the anatomic abnormalities of seminal vesicles in ADPKD and their clinical significance will be discussed.

Key Words: Autosomal dominant polycystic kidney disease; Megavesicles; Seminal vesicles; Seminal vesicle cysts

Introduction

Autosomal dominant polycystic kidneys disease (ADPKD) is the most common inherited kidney disease and is the fourth most common cause of end stage kidney disease. Extra-renal manifestations are prevalent, including cysts in liver, pancreas, spleen, and intracranial saccular aneurysms (1-3). Mutations in *PKD1* and *PKD2* genes are known to cause ADPKD, with *PKD1* mutations accounting for 75%-85% of cases. Polycystin-1 and polycystin-2 are integral membrane proteins encoded by the *PKD1* and *PKD2* genes, respectively. Our understanding of the role of these proteins in normal physiology and in the pathophysiology of ADPKD is evolving (2-4).

Few studies have evaluated ADPKD manifestations in the male reproductive system. These include case reports and studies reporting cystic changes in the seminal vesicles, including reports focused on infertility (5-19). This review will highlight seminal vesicle abnormalities in ADPKD.

Seminal vesicles

The seminal vesicles are a pair of glands positioned posterior and inferior to the urinary bladder, and lateral to the vas deferens. Each vesicle consists of a single tube coiled on itself and joined to the distal portion of the vas deferens (ductus deferens) which becomes the ejaculatory duct. The two ejaculatory ducts immediately pass through the prostate gland to open separately into the verumontanum of the prostatic urethra (Figure 1).

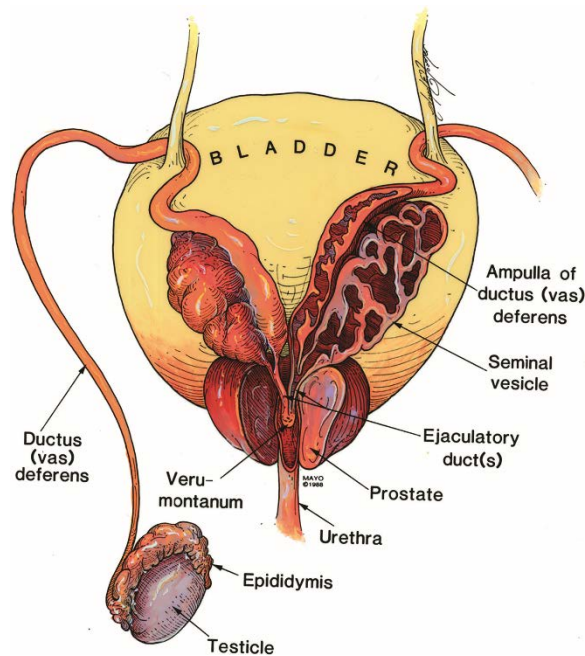


Figure 1. Posterior view of male urogenital organs including the seminal vesicles, vasa deferens, and ejaculatory ducts (Image purchased from the original copyright holder; 21).

The seminal vesicles produce and secrete seminal fluid, which consists of 50-80% of the ejaculate volume. The normal seminal vesicles are tortuous tubular fluid-containing structures with thin septa identified by ultrasonography (US), computed tomography (CT), and magnetic resonance imaging (MRI) (Figure 2). US is commonly used as initial choice of evaluation, whereas MRI is used for more complex cases. The normal mean seminal vesicle diameter is 1.5 ± 0.4 cm (by CT) (20, 21) or 0.9 ± 0.3 cm (by US) (22), and about 3.1 ± 0.5 cm in length (by CT) (21).

Anatomic abnormalities of seminal vesicles in ADPKD patients

Seminal vesicle cysts

Seminal vesicle cysts can be acquired or congenital. Acquired seminal vesicle cysts occur in the general population and are thought to be due to post-infection fibrosis or compression of the ejaculatory ducts related to obstructive causes (23). Congenital seminal vesicle cysts may occur as an isolated finding, but are predominantly associated with urogenital

anomalies, such as ipsilateral renal agenesis or dysgenesis, ectopic ureteral insertion, and vas deferens agenesis (21, 24, 25). Most studies of ADPKD patients reported bilateral seminal vesicle cysts (6-8, 17), although it is uncertain whether these cysts were acquired or congenital.

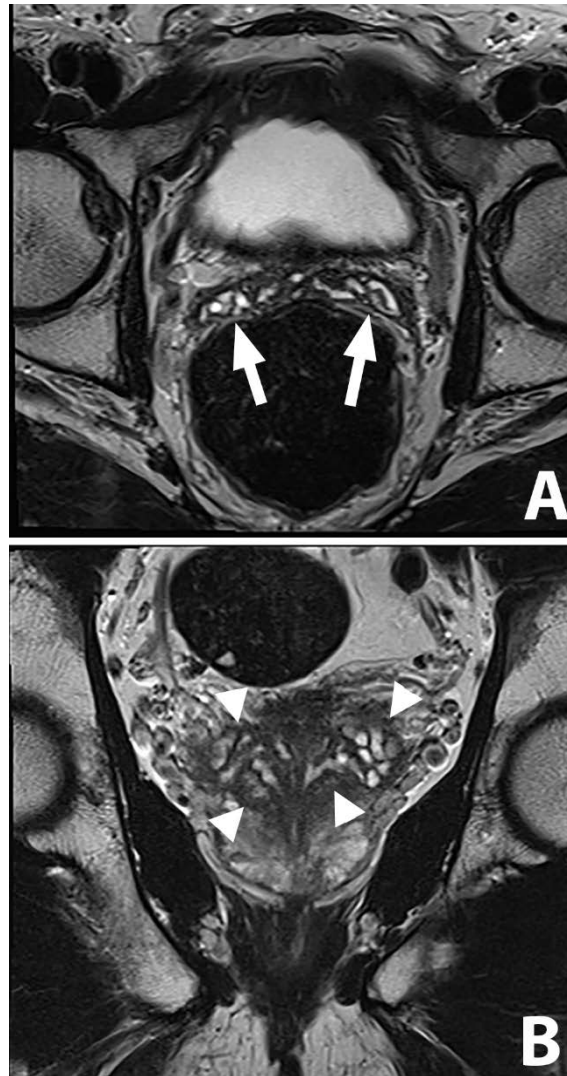


Figure 2. MR imaging appearance of normal seminal vesicles. Figure 2A. Axial T2-weighted image demonstrating tubular structures (arrows) with septae, representing normal seminal vesicles, posterior to the urine-filled bladder. Figure 2B. Coronal T2-weighted image showing seminal vesicles (arrowheads). Prostate gland is partly visualized inferior to the seminal vesicles.

Seminal Vesicles in ADPKD

The reported prevalence of seminal vesicle cysts in ADPKD patients varies widely using different imaging modalities; from 6% by CT (5), to 21% to 60% by transrectal US (6, 7, 17). However, the reported prevalence is 5.2% by transrectal US in the general population without ADPKD (26). Seminal vesicle cysts were identified by US as discrete anechoic areas (simple cysts) or hypoechoic areas containing internal echoes (hypoechoic cysts), measuring greater than 5 mm in diameter (6, 7, 17).

In a prospective study of ADPKD patients, Belet et al. reported a seminal vesicle cyst prevalence of 39% (41 out of 104 patients) using US studies (abdominal, transrectal, and scrotal); the prevalence was 2% in non-ADPKD controls (1 of 62 patients); $p < 0.01$ matched for age, level of renal function, and renal replacement therapy (6). Reproductive tract cysts were identified during evaluation of primary infertility (5%) or were found incidentally. In that study, 76% of the cysts were anechoic (simple) and 24% were hyperechoic. Cysts in the other regions of the reproductive tract were also identified in the 41 patients with seminal vesicle cysts; epididymal cysts were found in 20%, and prostatic cysts in 15%. There was no association of seminal vesicle cysts with cysts in the liver, epididymis, prostate, patient age or serum creatinine level.

In another prospective study of 28 ADPKD patients (age 18-50 years with estimated GFR > 60 ml/min/1.73 m²), Torra et al. reported seminal tract cysts in 10 patients (35.7%) and seminal vesicle cysts in 21%, using transrectal US (17). Eight patients (28.6%) in that study were being evaluated for infertility.

In a study of 45 ADPKD patients with a mean age 40 years, Danaci, et al. found the prevalence of seminal vesicle cysts to be 60%. None of the control subjects, who were matched for age, renal function, and renal replacement therapy, had seminal vesicle cysts (8). This study suggested that seminal vesicle cysts may develop later in life, based on their findings of a positive correlation between seminal vesicle cysts, hepatic cysts and serum creatinine concentrations. However no significant relationship between the presence of seminal vesicle cysts and the age of the patients was identified.

Seminal vesicle ectasia

Seminal vesicle cysts are the most commonly reported finding in the reproductive system of ADPKD patients (6-8, 17). However, seminal vesicle ectasia is a separate entity, which can be mistaken for cysts on US and other imaging modalities (14, 16-18). Dilatation of seminal vesicles has been demonstrated on ejaculatory ductograms (16). "Megavesicle" is defined as asymmetric or bilateral diffuse enlargement of anteroposterior dimension exceeding 1.0 cm, without isolated cysts or septa (Figure 3 and Figure 4). This entity must be distinguished

from seminal vesicle cysts, which are defined as isolated cysts adjacent to normal-diameter remaining parts of the seminal vesicles.

In a retrospective case-control study, Joo et al. compared the size of seminal vesicles in 68 ADPKD patients and 68 controls, matched for age using 3D CT (11). The mean diameter of seminal vesicles in the ADPKD group was significantly larger than the controls (axial: 1.70 vs 1.53 cm, $p=0.01$; coronal: 1.86 vs 1.68 cm, $p=0.02$); the diameter decreased with age in controls, but there was no association with age in the ADPKD group in this or other studies (11, 27). Interpretation of this study was limited by the absence of clinical information, including level of kidney function and prevalence of infertility.

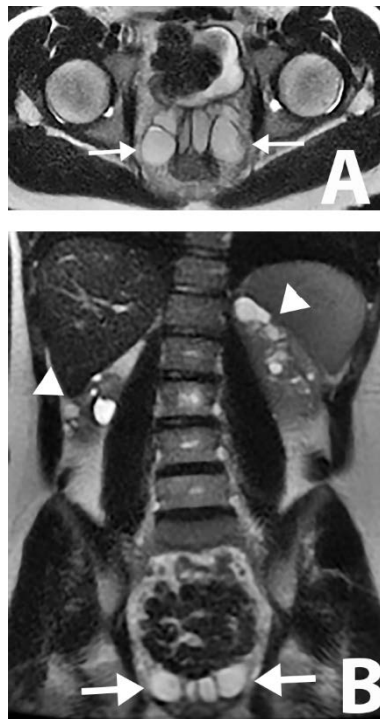


Figure 3. An example of abnormal seminal vesicles. A 19 year old male, diagnosed with ADPKD on ultrasound, without family history. Bilaterally dilated hyperintense seminal vesicles on single shot fast spin echo (SSFSE) images (axial and coronal images) on 1.5 T MR, measuring 1.9 cm on the left and 2.1 cm on the right. No reproductive symptom was reported at the time of imaging and no fertility evaluation was performed. Normal serum creatinine. A, Axial SSFSE image; B, Coronal SSFSE image, demonstrating dilated both seminal vesicles (arrows) and multiple cysts in both kidneys (arrowheads).

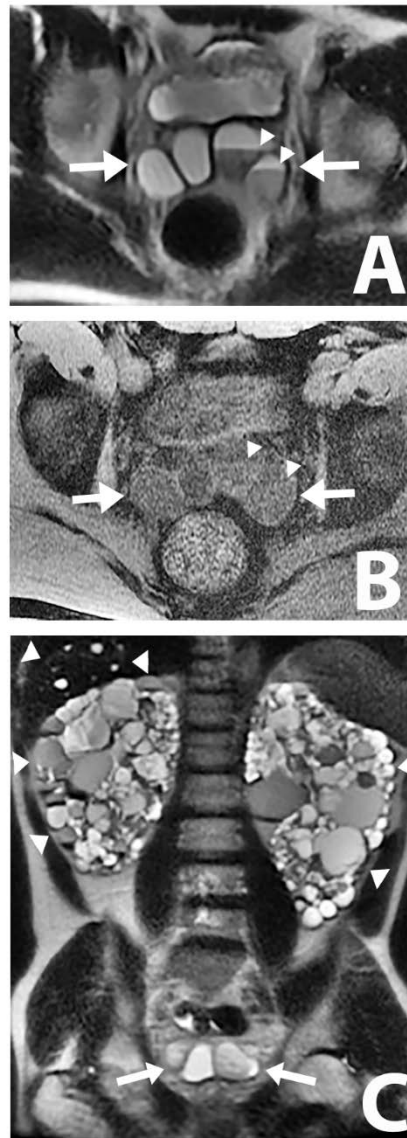


Figure 4. An example of abnormal seminal vesicles. A 47 year old male, with family history of ADPKD presenting with gross hematuria. Bilateral seminal vesicles are dilated measuring 3 cm, each with fluid level within the left seminal vesicle, possibly representing hematospermia. The patient has children. A, Axial SSFSE image; B, Axial T1 weighted LAVA image demonstrating high signal intensity fluid level in the left seminal vesicle, representing hematospermia; 4C, Coronal SSFSE image, demonstrating dilated seminal vesicles (arrows) as well as multiple cysts in the liver and kidneys (arrowheads).

Reig et al. reported a greater mean seminal vesicle diameter, measured by MRI, in ADPKD patients than in controls (4.2 mm vs 3.1 mm; $p < 0.0001$) (15). Moreover, in that study, the prevalence of megavesicles was 23% when a partition value of 1 cm diameter was used, and 15% when the partition value was 1.5 cm diameter. Dilated seminal vesicles can also rarely be found in patients without ADPKD, usually due to obstruction, which may be related to urogenital anomalies or calculi. These patients can present with hematospermia (14, 28, 29).

Megavesicles in ADPKD are reportedly not caused by obstruction (10). Hendry et al. identified free flow of dye in the ejaculatory duct on the percutaneous vesiculography, as well as free efflux of dye from the vesicles during surgery. The presence of sperm in the aspirate from the vesicle, and lack of improvement after ductal ostia resection in 3 of 4 patients, supports a nonobstructive cause of the megavesicles. Hendry et al. suggested that the underlying problem may be failure of propulsion of the contents within the seminal vesicles and ampullary parts of the vasa, rather than mechanical obstruction (10).

Although most of studies report a high prevalence of seminal vesicle cysts (up to 60%) in ADPKD, a large fraction of those cysts may actually be megavesicles that resemble cysts by ultrasonography (9, 10, 15). The advantages of dynamic study by vesiculography and superior soft tissue contrast MRI have likely resulted in the higher detection of megavesicles in the ADPKD population (Figures 2 and 3).

Clinical significance and infertility

Patients with ADPKD are generally known to be fertile, although there are several reports of male infertility in ADPKD. Torra et al. found that 28.5% of 28 ADPKD patients complained of infertility (17). This is a higher prevalence than in the general population, which is estimated as 15% in couples and 7% in men in western countries. However, Belet et al. reported only 5% prevalence of infertility in a prospective study of 104 ADPKD patients based on patient interviews, which was a larger ADPKD patient group than that reported by Torra et al. (6).

Included among the potential etiologies of male infertility in ADPKD are: (i) uremia; (ii) necropermia (low sperm motility with high proportion of dead sperm); (iii) immotile sperm that have been attributed in some cases to ultrastructural flagellar defects caused by abnormal polycystins; (iv) seminal vesicle cysts; and (v) ejaculatory duct cysts (6, 17-19, 30).

There are few studies of infertility and seminal vesicle abnormalities in ADPKD patients (6, 9, 10, 12, 17, 31). Belet et al. noted presence of delayed liquefaction and hyperviscosity in one semen analysis of an infertile patient, and suggested that the seminal vesicle cyst formation caused infertility (6). Hendry et al. proposed that failure of seminal vesicles to contract effectively caused megavesicles and eventually led to decreased ejaculate volume (10). Fang et al. reviewed data obtained from 4,108 infertile men for necrospemia and found 20.7% of the infertile patients with necrospemia had ADPKD (9), with one case demonstrating dilated seminal vesicles by US. They speculated that necrospemia in ADPKD may result from stasis of the seminal tract content due to functional obstruction, and subsequent sperm death and degeneration (9).

Torra et al. reported prevalence of semen abnormalities and cysts in the seminal tract (17). A semen analysis, performed on 28 ADPKD patients, showed abnormalities in 91% and decreased seminal volume in 30% of ADPKD patients. Although decreased seminal volume may be related to distal seminal tract obstruction due to extrinsic compression by seminal vesicle cysts or an abnormality within seminal vesicles, no significant association between seminal tract cysts and semen abnormality was noted in that study. Torra et al. suggested that the presence of seminal tract cysts likely has limited, if any, clinical consequences, given the relatively high prevalence of seminal tract cysts and the relatively low frequency of infertility in ADPKD (17).

The most common semen abnormality in the study by Torra et al. was asthenozoospermia (reduced sperm motility) (7). When compared to semen donors without ADPKD, the percentage of progressive motile forms was the only significant difference ($p < 0.0001$) (17). This suggests that an abnormality in polycystins in ADPKD which play a role in the abnormal structure of the cilia or flagella may affect sperm motility (12, 17, 31).

Summary and conclusion

ADPKD is associated with cystic dilatation and ectasia of seminal vesicles. The pathogenesis of these abnormal findings and their relation to infertility is controversial. Prospective, controlled studies of the male reproductive tract and sperm ultrastructure and functional characteristics are required to define the prevalence and mechanisms of infertility in men with ADPKD.

Conflict of interest

The authors declare that they have no conflicts of interest with respect to research, authorship and/or publication of this book chapter.

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Chapter 19

Craniofacial Development and Growth in Polycystic Kidney Disease

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Abstract

Autosomal dominant polycystic kidney disease (ADPKD) is an inherited disorder characterized by the presence of multiple cysts in kidneys. ADPKD has been shown to be caused by mutations in the genes of PKD1 and PKD2, encoding polycystin-1 (PC1) and polycystin-2 (PC2), respectively. Polycystins are localized in primary cilia that play roles in multiple biological processes including mechanoreception, Ca²⁺ influx and cell signalling

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pathways. Primary cilia are known to play important roles in regulating craniofacial development and growth. In this chapter, we summarize the function of Pkd1 and Pkd2 in controlling mouse craniofacial development and growth, and discuss PKD-associated molecular mechanisms.

Key words: Craniofacial; Development; Postnatal growth; Sutures

Introduction

The vertebrate head is the most complex structure of a body, containing diverse organs such as the brain, bone, eye, ear, nose, tongue and teeth. Head development and growth require the co-ordination of multiple processes in time and space and many signaling pathways such as fibroblast growth factor (FGF), sonic hedgehog (Shh), bone morphogenetic protein (Bmp) and Wnt are known to play critical roles in these processes. Other processes such as mechanical force can also affect craniofacial development and growth. Mutations in the *PKD1* (encodes Polycystin-1/PC1) and *PKD2* (encodes Polycystin-2/PC2) genes have been shown to account for autosomal dominant polycystic kidney disease (ADPKD), which is characterized by the presence of renal and extra-renal cysts, cardiovascular abnormalities including hypertension and intracranial aneurysms. In this chapter, we discuss recent evidence that identifies an intriguing link between polycystic kidney disease and head development and growth.

The mammalian head

The head is derived from all embryonic germ layers: ectoderm, mesoderm, endoderm and neural crest-derived ectomesenchyme. Neural crest cells undergo epithelial-mesenchymal transition, migrate to different locations, and differentiate into multiple cell types in the embryo (1,2).

The human skull consists of twenty-two bones, eight bones of the neurocranium that surround the brain and brainstem, and fourteen bones of the viscerocranium that supports the face (3, 4). Mesenchymal cells forming jaw and facial bones are derived from neural crest cells. The frontal bones, the medial part of the interparietal bone, and tooth-supporting tissue, alveolar bone and periodontal ligament tissue are also of neural crest origin. The parietal and the lateral parts of the interparietal bones are of mesodermal origin in mice (5, 6).

Cranial bones abut at sutures that comprise a fibrous tissue between the bones. The skull mostly enlarges along the suture margins by bone deposition. Slight movement is permitted at sutures, when the suture is still open, which is important for skull development and growth. Abnormalities in ossification in these sutures lead to craniofacial deformities (4, 7). Sutures are not just vital for the skull, but also brain development and growth, that should accommodate the growing brain (3, 4, 7).

The cranial base is also an important growth center of the craniofacial skeleton where growth is controlled by synchondroses that involve the formation of either hyaline cartilage or fibro-cartilage between craniobase bones (1, 4). A synchondrosis usually temporary exists during the growth phase, and is ultimately converted into bone. Cranial base synchondroses are termed ethmoidal, intrasphenoidal, sphenoccipital or intraoccipital according to their anatomical location. Abnormalities in cranial base synchondroses result in craniofacial deformities (1, 4). In mice, both the presphenoid and phenooccipital synchondroses remain patent in adulthood (8, 9).

Conditional deletion of *Pkd1/Pkd2* in mice

Gene targeting in mice (knockout mice) is a powerful tool to determine the function of genes during development and growth. It is however difficult to investigate the relationship between *Pkd1* or *Pkd2*, and head development or growth using global *Pkd1* or *Pkd2* null mutant mice, since homozygosity for the *Pkd1* or *Pkd2* null alleles leads to early embryonic lethality between embryonic day (E) 13.5 and parturition (10, 11).

The Cre/loxP system is a commonly used tool to alter the mouse genome in a conditional manner (site- and time-specific) by deletion of loxP-flanked DNA segments. Cranial neural crest cell-specific gene targeting has been achieved by *Wnt1*-driven Cre recombinase to investigate the molecular mechanisms in craniofacial development (6, 12, 13). *Dermo1Cre* mice have been used for gene targeting skeletal tissues (14, 15, 16). *OsxCre* and *Col2a1Cre* ER* strains can be used for deletion of the genes from osteoblasts and chondrocytes, respectively.

Mice with conditional *Pkd2* inactivation using *Wnt1Cre* (*Pkd2^{fl/fl};Wnt1Cre*) survive until adulthood and show a shortened snout, malocclusion, a dome-shaped skull vault and curved spine (Figure. 1) (17). Mice with conditional deletion of *Pkd1* in neural crest-derived cells (*Pkd1^{fl/fl};Wnt1Cre*) show similar craniofacial phenotypes to those seen in *Pkd2^{fl/fl};Wnt1Cre* mice (15, 18). Since no significant morphological craniofacial changes can be observed either in *Pkd1^{fl/fl};Wnt1Cre* or *Pkd2^{fl/fl};Wnt1Cre* mice at birth, it is likely that the anomalies in mutant heads occur after birth.

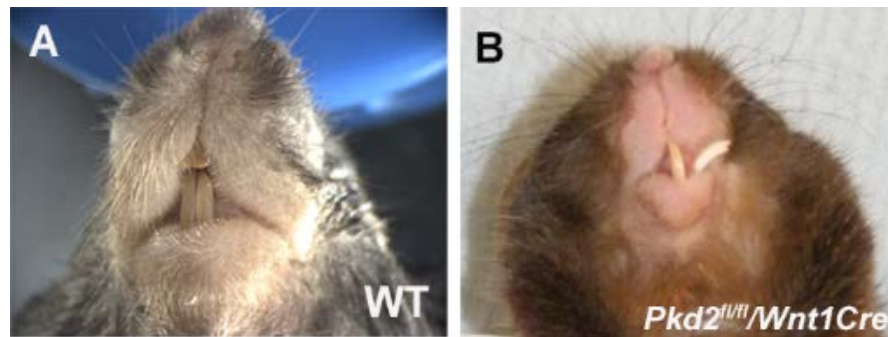


Figure 1. Adult heads of wild type and *Pkd2* conditional knockout mice showing abnormal incisor formation/occlusion. A, wild-type; B, *Pkd2^{fl/fl};Wnt1Cre* mice.

Mechanical stress and *Pkd1/Pkd2* in mastication

Mastication produces strong and frequent mechanical stresses that are perceived by teeth, the periodontal ligament and the temporomandibular joints. *Pkd2^{fl/fl};Wnt1Cre* mice show fractures and dilacerations of teeth, alveolar bone loss and increased width of the periodontal ligament tissue. The temporomandibular joints are also compressed in *Pkd2* mutants (17). Although low levels of calcification of dentin or alveolar bone could not be detected in mutants, these results still suggested that abnormally strong occlusal forces were occurring in *Pkd2* mutants.

Mastication, including occlusion and jaw movements, is coordinated by the interaction between teeth, periodontal ligament, skin, muscle, mucosa and the temporomandibular joints. The strength of the occlusal force is known to be controlled by the central nervous system and feedback of the mechanical stress from many tissues such as periodontal tissues. Mechanical sensation can evoke the excitation or inhibition of various oral reflexes to facilitate mastication by the controlling activity of jaw-closing muscles. Inhibitory reflexes are known to be the predominant response to avoid the excess forces that would destroy tissues. Perception in the periodontal ligament has been shown to play a critical role in providing a substantial part of the information of mechanical sensation. The temporomandibular joints are also believed to control mastication through perception of the occlusal force (19). *Pkd2* is deleted only in neural crest-derived cells in *Pkd2^{fl/fl};Wnt1Cre* mice. Neural crest cells differentiate into periodontal ligament and the temporomandibular joints, but not into the central nervous system or muscle, suggesting that abnormal occlusion is caused by abnormal perception in the periodontal ligament and/or the temporomandibular joints due to *Pkd2* mutation.

Mechanical stimuli such as occlusal force are believed to be detected by mechanoreceptors. Primary cilia are non-motile microtubule-based organelles that are found on most cells. Primary cilia are known to sense fluid flow in many tissues including the lumen of renal tubules and blood vessels (20). Both PC1 and PC2 are localized at the plasma membrane of the primary cilia, and PC1 has been shown to function with PC2 to act as a mechanoreceptor in primary cilia in kidneys and blood vessels (21).

Primary cilia are found in cells of periodontal ligament tissues and the temporomandibular joints. The bending of primary cilia in the periodontal ligament and temporomandibular joint cells may regulate a feedback of mechanical stresses to the central nervous system to control further occlusal force. It is possible that the changes by mutation of either *Pkd1* or *Pkd2* are related to the loss of function of primary cilia as mechanocensors in periodontal ligament and/or the temporomandibular joints, which induce abnormal occlusal force.

Mechanical force and head morphology

Bone is constantly remodeled by the coordinated action between bone-resorbing osteoclasts and bone-forming osteoblasts throughout life. This continuous remodeling serves to prevent and remove fatigue-related micro-damage and allows adaptation of the bone mass and structure. The balance between the amount of bone resorption and bone formation is determined by many factors including mechanical forces. Exercise results in enhanced bone formation, whereas bone loss is observed in the absence of mechanical stimulation including immobilization, disuse and exposure to low gravity. Increased mechanical stress thus stimulates bone formation.

The fibrous joints in cranial bones and the cranial base play a critical role in enlargement of the skull vault, and premature fusion by abnormal ossification in one or more cranial sutures leads to an abnormal head shape such as a dome-shaped skull. Abnormal fusion of front-maxillary and front-nasal sutures are observed in *Pkd2^{fl/fl};Wnt1Cre* mice. Moreover *Pkd1^{fl/fl};Wnt1Cre* and *Pkd2^{fl/fl};Wnt1Cre* mice show abnormal anterior synchondrosis in pre-sphenoid and intra-sphenoid (17, 18). Loading *in vivo* and *ex vivo* skull models results in abnormal closure of sutures (22). Mechanical stresses caused by mastication and jaw movement are known to affect craniofacial morphogenesis during postnatal growth (23, 24, 25, 26). These studies suggest that abnormal mechanical force by traumatic occlusion can alter the balance between bone resorption and bone formation in sutures in *Pkd1* and *Pkd2* mutants, resulting in premature fusion and subsequently morphological skull abnormalities including a dome-shaped skull and anterior-posterior compression of the snout.

The relationship between *Pkd1/Pkd2* and mechanical force on bone formation

The neural crest contributes to anterior cranial base sutures including the pre-sphenoid, whereas posterior cranial base sutures including phenoooccipital synchondrosis are formed by mesodermal cells. *Pkd1*^{fl/fl};*Wnt1Cre* and *Pkd2*^{fl/fl};*Wnt1Cre* mice showed only abnormal anterior synchondrosis in pre-sphenoid and intra-sphenoid, while *Pkd1* conditional deletion using Cre recombinase regulated by the promoter of the mesoderm-specific *Dermo1* showed the formation of a ossified bridge in the phenoooccipital synchondrosis. Mesoderm conditional deletion of *Pkd1* resulted in a delay in ossification of the axial and appendicular skeleton. *Pkd1* and *Pkd2* thus play roles in both membranous (neural crest) and enchondral (mesoderm) ossification.

Mechanical stress has been shown to regulate the differentiation and proliferation of chondrocytes and osteoprogenitor cells (27, 28, 29). *Pkd1* is expressed in prechondrocytes, osteoblasts and osteocytes (30, 31). In order to clarify the interaction between *Pkd1* and mechanical force on ossification in sutures, midpalatal suture expansion has been studied as a mechanical force (32) and shown to lead to new bone formation. Osteogenic response to tensile stress is significantly reduced due to reduced proliferation, delayed differentiation and increased apoptosis of osteochondroprogenitor cells in *Pkd1* mutants. Cellular responses have been shown to vary dependent on the magnitude of mechanical force, and its frequency or duration (33, 34, 35). In addition, the stage of cellular differentiation is also likely to be involved in cellular responses to mechanical force (32). At a physiological level of mechanical stress, *Pkd1* negatively regulates the differentiation of osteoprogenitor cells to osteoblasts, whereas it accelerates differentiation to matured osteoblasts. *Pkd1* also induces matrix production by mature osteoblasts. *Pkd1/Pkd2* mutations thus directly alter the response of osteoprogenitor cells to mechanical force.

The back-and-forth movement of extracellular fluid in the bone microenvironment during exercise has been shown to bend, deform or stretch the primary cilia of osteocytes (36). Abnormal function of primary cilia due to *Pkd1/Pkd2* mutation in osteogenic cells and/or abnormal bending of the primary cilia due to traumatic occlusal force may affect bone formation in mutant skulls.

Mechanical stress also alters cell shape and cellular structures that change the influx and efflux of ions. Calcium ions play critical roles in many biological processes including exerting allosteric regulatory effects on many enzymes and proteins, and acting as a signal transducer. Conformational changes in the polycystin complex have been shown to result in Ca²⁺ entry (37). *Pkd1* and *Pkd2* mutations are known to lead to

abnormal Ca^{2+} influx (20, 37, 38). Interestingly, intracellular calcium concentration is elevated in periodontal ligament cells when they receive hydraulic pressure (39). Aberrant occlusal force is likely to change Ca^{2+} influx, which may affect bone formation in the skull.

Extracellular domains of PC1 and PC2 have been shown to play a role in cell-cell and/or cell-extracellular matrix interaction by formation of multiprotein complexes with integrins and other focal adhesion proteins including paxillin, talin, tensin, focal adhesion kinase and c-Src (20, 40, 41). Focal adhesion involving integrins is known to play a role in head development (42, 43). These complexes are often clusters of large macromolecular assemblies that transmit mechanical force and may be affected by *Pkd1/Pkd2* mutations.

Mechanical stress-independent *Pkd1/Pkd2* function

Although newborn heads show no significant changes in *Pkd1/Pkd2* mutants, intramembranous ossification of the skull is slightly delayed in *Pkd1^{fl/fl};Wnt1Cre* at birth (17, 18). Small excess bone deposition is also observed along the sutures of the snout in newborn *Pkd2^{fl/fl};Wnt1Cre* mice. Mesoderm conditional deletion of *Pkd1* also leads to enlarged cranial base fenestrae.

Bone-like structures are observed in *Pkd2* mutant dental pulp that is isolated from any obvious external force (17). Reparative dentin is formed in the pulp underneath dental caries or thin dentin as a reaction to external stimuli affecting odontoblasts. Odontoblasts are tall columnar cells with a polarized distribution of their cytoplasmic organelles. Reparative dentin often has a bone-like structure and is formed by odontoblasts that lose their polarity. Cells with no columnar shape are found to surround bone-like structures observed in *Pkd2* mutant dental pulp, although there is no sign of dental caries or thin dentin. Runx2 is upregulated in *Pkd2* mutant dental pulps and mice with overexpression of Runx2 also show bone-like structures in dental pulp (17, 44). Upregulation of *Runx2* is also observed in the nasal cartilage of *Pkd1* mutants, which shows abnormal ossification. These results suggest that *Pkd2* mutation leads to abnormal differentiation or function of odontoblasts. *Pkd1* and *Pkd2* may thus possess mechanical force-independent functions at embryonic stages, during growth, and into adulthood. Knockdown of the primary cilia protein, Polaris, resulted in abrogation of Runx2 (45) and since primary cilia are also present in the dental pulp, it is possible that the mechanical force-independent function of *Pkd1* and *Pkd2* is related to primary ciliary function.

Craniofacial characteristics of ADPKD patients

Experimental analysis of the roles of Pdk1 and Pdk2 in craniofacial development and growth in mice suggests that mutations of these genes in humans that give rise to kidney abnormalities, may also affect craniofacial characteristics. Rapid head growth with a bulging anterior fontanelle and sutural separation has been reported in a patient with ADPKD due to *PKD1* mutation (46). Three-dimensional photography combined with dense surface modelling was performed on ADPKD patients to analyze face morphology. Dense surface shape analysis showed individuals with ADPKD to have vertical lengthening of the face, predominantly in the upper third, and mild mid-facial hypoplasia. Linear measures derived from 3D facial landmarks showed individuals with ADPKD to have a moderate lengthening of the nose confirmed by an increase in the ratio of nasion-pronasale:nasion-subnasale (17). Although further studies are needed, it is possible that faces of ADPKD patients show subtle characteristic facial features. Since *Pkd2* mutant mice data show the possibilities of excess occlusal force, craniofacial structures may perceive supraphysiological mechanical stress in ADPKD patients.

The anatomy of the human face is different from mice and includes the presence of maxillary sinuses for example. These different structures might modify the distribution of constraints on the face. It is likely that the distribution of mechanical-related anomalies is different between humans and mouse mutants (47). These may cause different facial phenotypes between mutant mice and patients. On the other hand, in common with mutant mice, a shift of the mid-face is observed in patients (17). That is consistent with the fact that the mid-face is a susceptible region for mechanical stress during growth (22).

Conclusion and perspective

Emerging evidence suggests that *Pkd1/Pkd2* mutations are responsible not only for kidney disease, but also for craniofacial anomalies in mice and specific facial characteristics in humans. Wnt and Shh signaling pathways are known to regulate craniofacial development and since primary cilia are involved in activation of both pathways, the function of *Pkd1/Pkd2* in craniofacial development and growth may be related to Wnt and Shh pathways (48, 49). Further studies are required to clear the precise role of *PKD1* and *PKD2* in craniofacial development and growth.

Conflict of interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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Chapter 20

Rapidly Progressive Glomerulonephritis in Autosomal Dominant Polycystic Kidney Disease

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Abstract

Patients with autosomal dominant polycystic kidney disease (ADPKD) can suffer from the same causes of acute kidney injury as the general population. Affected individuals may present with hematuria and proteinuria (usually less than 1g/day). However, nephrotic syndrome and proliferative glomerulonephritis are uncommon in patients with polycystic kidney disease. Development of nephrotic syndrome and / or rapid deterioration in kidney function suggest the presence of another, more aggressive disorder, requiring

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prompt diagnosis and appropriate interventions to mitigate further injury and progression to end stage kidney disease. In this chapter, we will discuss rapidly progressive glomerulonephritis in association with ADPKD.

Key Words: Acute Kidney Injury; Polycystic Kidney Disease; Rapidly Progressive Glomerulonephritis

Introduction

Polycystic kidney disease is the most common cause of monogenic inherited kidney disease and is associated with extra-renal manifestation of cystogenesis primarily in liver and pancreas. It is also associated with saccular aneurysm in the central nervous system (CNS) vasculature, and mitral valve disease (1). Amongst all cystic kidney disorders, autosomal-dominant polycystic kidney disease (ADPKD) is the most frequently diagnosed, affects more than 12 million people worldwide and accounts for about 7-10% of all patients with end stage kidney disease (ESKD) (2). In the majority of cases, the genetic basis for ADPKD has been identified as mutations in one of two large proteins, polycystin 1 and 2 coded by genes *PKD1* and *PKD2* located on chromosome 16 and chromosome 4, respectively (for review, please refer to references (3;4)). There is currently no evidence to support the possibility of mutations in other gene(s) accounting for ADPKD. Ultrasonographic evaluation remains the primary tool for diagnosis because it is inexpensive and widely available. The age-dependent criteria for diagnosis or exclusion of ADPKD have been recently reviewed (5). For patients less than 40 years of age, ultrasonographic criteria may be insufficient to exclude ADPKD. In these circumstances, magnetic resonance imaging (MRI) has proven valuable. A finding of fewer than five kidney cysts by MRI is sufficient to exclude ADPKD (6). Hence, in most cases, genetic testing is not routinely performed for diagnosis of ADPKD.

The phenotypic manifestation of those with mutations in *PKD2* is milder than that seen in individuals with *PKD1* mutations; the former presents with fewer kidney cysts, later onset of hypertension, chronic kidney disease (CKD) and ESKD and the overall survival is better (7). The penetrance of both mutations is highly variable in that cysts and chronic kidney disease may develop at different ages within the same family with identical mutation. The reasons for this are unclear and various hypotheses have been advanced to address this observation (8-10). The 'two-hit' model of cystogenesis has been most widely utilized to explain cystogenesis in ADPKD. The model contends that a germ-line mutation combines with somatic mutations in epithelial cells to promote cyst formation; in this model somatic mutation would be the rate limiting step for cystogenesis (8). A 'three-hit' model of

cystogenesis, in which kidney injury is the third hit, has also been proposed (10). Both of these models are not universally accepted as explanation for variability in cystogenesis and alternative models are being evaluated.

Progressive development of kidney cysts and associated increase in kidney volume are features of ADPKD. Other manifestations of kidney disease include sub-nephrotic range proteinuria, infected cysts associated with constitutional symptoms, and microscopic or macroscopic hematuria (with or without flank pain). Nephrotic range proteinuria (protein excretion > 3.5g/day) is an uncommon occurrence. Whereas development and progression of CKD occurs earlier in individuals with *PKD1* mutation, kidney function may be normal for decades and is not considered a very reliable marker of disease burden. Total kidney volume (TKV) in relation to age provides an accurate estimate of cyst burden and has been associated with hypertension, hematuria and progression of CKD (11;12). Acute renal failure secondary to rapidly progressive glomerular nephritis is rare in those with polycystic kidney disease. In the following sections, we will review the topic of acute renal failure in association with ADPKD. We will start by reviewing a case of rapidly-progressive glomerulonephritis (RPGN) in a patient with ADPKD.

Case

A 43-year-old man, without significant medical or surgical history, presented to the emergency department complaining of bilateral lower extremity edema for two weeks. The patient denied any pertinent family medical history. Physical exam was notable for pitting edema in his lower extremities. Laboratory investigations revealed hematuria, nephrotic range proteinuria (25g/24 hours) and kidney dysfunction (serum creatinine 2.6 mg/dL). Abdominal ultrasound was notable for bilaterally enlarged kidneys with multiple cysts; these findings were confirmed by computed tomographic (CT) scanning. Based on ultrasonographic criteria (age 40-59 years with two or more cysts in each kidney) (5), the patient was diagnosed with ADPKD. A complete serologic evaluation, including hepatitis screen, serology for HIV, hepatitis B and C, syphilis screen, c-anti-neutrophil cytoplasmic antibody (c-ANCA), p-ANCA, anti-GBM anti-nuclear antibodies (ANA) and anti-dsDNA, was performed and was unrevealing. Complements (C3 and C4) levels were within normal limits. Serum and urine protein electrophoresis did not reveal any monoclonal proteins. Initially, empirical treatment with intravenous steroid (methyl prednisolone, 1g daily x 3 days) was administered, then therapy was switched to oral steroid (prednisone, 1 milligram per kilogram daily). Notwithstanding ongoing therapy with prednisone (1mg/kg), renal function declined. Given development oliguria, progressive uremic symptoms and anasarca that was unresponsive to diuretic therapy, intermittent

hemodialysis was initiated. A CT-guided kidney biopsy revealed immune complex glomerulonephritis (positive IgG and C3 on immunofluorescence) with crescent formation and features of membranous nephropathy and (13). Therapy with prednisone was continued and, in addition, treatment with mycophenolate mofetil (500mg, twice daily; escalated to 1500mg twice daily over 2 weeks) was initiated. In order to augment the management of blood pressure and proteinuria, the patient was also treated with Lisinopril (40 mg daily) to manage blood pressure and proteinuria. Over a five-month period, the patient manifested improved kidney function, decreased proteinuria (from 25g to 3g daily), improvement in serum albumin normal levels and increased urine output. Given persistent nephrotic-range proteinuria, oral cyclosporine (75mg twice daily) was added to his treatment regimen. Over the ensuing 3 months, the patient manifested further improvement in physiologic parameters. Accordingly, prednisone dosage was reduced (10mg orally per day). Mycophenylate mofetil (1500 mg twice) and cyclosporine (75 mg twice daily) were maintained. Hemodialysis was subsequently discontinued and he has been off dialysis with stable CKD stage 3 (creatinine clearance 35-40mls/min by 24-hour urine studies) for greater than 2 years. He continues to be monitored closely in the outpatient setting for any changes in renal function. Immunosuppressive agents have been tapered.

Nephrotic syndrome in ADPKD

Nephrotic syndrome is characterized by urine protein excretion greater than 3.5 grams per 1.73 m² per day, low serum albumin level, high serum cholesterol level, and peripheral edema (14). Nephrotic syndrome in adults with polycystic kidney disease was initially reported about fifty-eight years ago (15). In that report, the frequency of nephrotic range proteinuria was 2.5% (3 patients amongst 122 cases). While early reports did not document biopsy evidence of kidney lesions, in general, nephrotic range proteinuria is an uncommon occurrence in persons with ADPKD. Indeed, over the subsequent forty years since the initial report of Dalgaard (15), about thirty-five cases of nephrotic syndrome with documented kidney pathology have been reported (16-18). When nephrotic syndrome has been reported in patients with polycystic kidney disease, the presentation (including nephrotic range proteinuria, edema, hyperlipidemia with and without hypertension), is similar to that seen in patients without polycystic kidney disease. Various etiologies have been identified for nephrotic syndrome in ADPKD patients including membranous glomerulonephritis (MGN), focal and segmental glomerulosclerosis (FSGS), minimal change disease (16;19;20) and immunoglobulin A (IgA) nephropathy (17;21). Of these, FSGS appears to be the most frequently reported cause of nephrotic range proteinuria in adults with ADPKD (16;18;22).

There are no established guidelines for therapeutic management of nephrotic syndrome in ADPKD. Accordingly, as with other cases of nephrotic syndrome, the mainstay of therapy involves angiotensin converting enzyme inhibitors or angiotensin II receptor blockers and corticosteroid therapy. However, a more aggressive immunosuppressive agent may be needed if proliferative lesions (for example, mesangioproliferative glomerulonephritis) are identified on kidney biopsy specimen. In reported cases, the responses of patients were dependent on extent of proteinuria, degree of kidney dysfunction and whether renal replacement therapy was needed (16;18;22).

Rapidly progressive glomerulonephritis

RPGN, also termed crescentic GN, is a pathologic entity primarily characterized by extra-capillary cellular proliferation in Bowman's space. Crescents, involving greater than fifty percent of glomeruli, are derived from epithelial cells and activated macrophages. Included in this group of diseases are small vessel vasculitis, Goodpasture's syndrome and ANCA associated vasculitis (23). In children and adolescents, Henoch-Schönlein purpura is more common while in women of childbearing age, systemic lupus nephritis is a more frequent cause of RPGN (24).

Three categories of RPGN have been described: type 1, presence of immune deposits in basement membrane; type 2, immune deposits in the mesangium and basement membrane; type 3, absence of glomerular immune deposit (23;25;26). Type 3 RPGN is the most common and accounts for about 50-60% of RPGN, Type 2 accounts for 15-20 %, and Type 1 is the least common (about 10% of RPGN). Type 1 RPGN primarily includes Goodpasture's syndrome (anti-glomerular basement membrane glomerulonephritis, anti-GBM GN) whereas Type 2 RPGN encompasses a diverse group of conditions including systemic lupus nephritis, IgA nephritis, Henoch-Schönlein purpura and immune complex mediated membranoproliferative glomerulonephritis. Type 3 RPGN includes microscopic polyangitis (MPA), granulomatosis with polyangitis (GPA) and eosinophilic granulomatosis with polyangitis (EGPA) (27).

Clinically, all patients with RPGN present with nephrotic syndrome and rapid decline in kidney function. In addition, extra-renal signs and symptoms (arthralgias, skin rash, pericarditis, peripheral neuropathy, rhinitis, sinusitis) may be present. Patients affected by RPGN are at high risk for progression to ESKD. Early diagnosis and therapeutic intervention is indicated to mitigate progression to ESKD. Renal biopsy is important for diagnosis, prognosis and to guide therapy.

Treatment is based on the combination of corticosteroids and cytotoxic agents (cyclophosphamide). In addition, plasma exchange (PLEX) is indicated in patients with Goodpasture's syndrome (28;29) and may be beneficial in resistant cases of type 2 RPGN. PLEX is indicated in patients with alveolar hemorrhage (30;31). Likewise, for patients with ANCA-associated vasculitis and RPGN, PLEX augments therapy with corticosteroids and cytotoxic agents and positively impacts patients' outcome and survival (32;33). Rituximab plus corticosteroids are as effective as cyclophosphamide for treatment of patients with type 3 RPGN (34;35). However, the effectiveness of Rituximab plus corticosteroid regimen as rescue therapy in resistant type RPGN disorders like lupus crescentic nephritis is unclear (36).

Proliferative glomerulonephritis in ADPKD

Crescentic glomerulonephritis is a rare occurrence in patients those with ADPKD. In addition to the case highlighted above (13), three additional cases have been reported (37;38); ADPKD diagnosis was based on ultrasonographic criteria in these cases. All cases were characterized by nephrotic range proteinuria, hematuria and acute kidney injury indicated by the rapid decline in kidney function. In the most recently reported cases (38), both patients had ANCA-associated crescentic glomerulonephritis without evidence of vasculitis. In the case we presented in this review, the cause of acute kidney injury and nephrotic syndrome was attributed to crescentic glomerulonephritis rather than the patient's underlying polycystic kidney disease.

The reasons for occurrence of glomerulonephropathies, with massive proteinuria, in ADPKD remain unclear. The three patients in the other reports (37;38) were older than our patient and one of the two patients developed ESKD and remained dialysis-dependent. However, common features or specific risk factors for development of RPGN amongst the four cases were unknown. In the current case, common causes of rapidly progressive kidney failure and nephrotic range proteinuria, including post-infectious causes, hepatitis B and C and IgA nephropathy were excluded. Given the limited number of cases of RPGN in patients with ADPKD, ascertaining mechanism(s) will be challenging.

All reported series emphasize the importance of performing kidney biopsies in patients with ADPKD who develop nephrotic range proteinuria. In addition to identifying the cause of proteinuria and acute kidney injury in these patients, renal biopsy informs appropriate management of proteinuria. In our patient, the biopsy findings of crescentic GN mandated a more aggressive, immunosuppressive regimen in management of the patient.

Table 1. Details on patients with PKD and crescentic Glomerulonephritis

Authors	Age (years)	Gender	Acute Kidney Injury	Nephrotic Range Proteinuria (g/day)	Hematuria (microscopic)	Renal Histopathology	Treatment	Outcome
Licina et. al. (37)	69	F	Yes	NQ / NR	Yes	CresGN	CS	Cr 2.4 mg/dL
Sumida et. al. (38)	60	F	Yes	Yes (4.5)	Yes	ANCA-MPO CresGN	CS	Cr 2.8 mg/dL Proteinuria (0.2g/day)
Sumida et. al. (38) CresGN	54	F	Yes	Yes (5.7)	Yes	ANCA-MPO CresGN	CS, PLEX	HD-dependent
Maggard et. al. (13)	45	M	Yes	Yes (25)	Yes	ICGN / MGN Cres-GN	CS, MMF CsA, ACEi	HD x 8 months Cr 2.6 Proteinuria (0.8 g/day)

ACEi: angiotensin converting enzyme inhibitor; ANCA: anti-neutrophil cytoplasmic antibody; NQ: Not quantified; NR: Not reported; CS: Corticosteroid; CsA – Cyclosporin; CresGN: Crescentic Glomerulonephritis; HD: Hemodialysis; ICGN: Immune complex glomerulonephritis; MPO: Myeloperoxidase; MMF: Mycophenolate Mofetil; PLEX Plasma Exchange.

Given the rarity of RPGN (or crescentic GN) in patients with polycystic kidney disease, there are no established guidelines on management. ADPKD in this patient was diagnosed on presentation and in view of the superimposed RPGN, management of the latter was the primary goal. The combination of steroid and mycophenolate mofetil was chosen over steroids and cyclophosphamide because of better side effect profile of mycophenolate mofetil. Cyclosporine was added to enhance management of proteinuria. The patient responded well to the combination of steroids, mycophenolate mofetil and cyclosporine. In the other reported cases of crescentic GN in patients with ADPKD, treatment included corticosteroids alone or in combination with PLEX (37;38). Like our patient, in two of the three reported cases, kidney function improved but did not return to normal (serum creatinine range 2.4 – 2.8 mg/dL); the third patient (one of two with ANCA-associated glomerulonephritis, without vasculitis) remains dialysis-dependent (Table 1).

Conclusions

Proteinuria (<1g/day) is not uncommon in individuals with polycystic kidney disease but nephrotic range protein excretion (>3.5g/day) is an infrequent occurrence. Intermittent hematuria in adults with polycystic kidney disease, without significant changes in urine protein loss may reflect cyst hemorrhage. This is more likely if hematuria is accompanied by flank pain without other constitutional symptoms (like fever) that would suggest an infectious process. The development of nephrotic range proteinuria in association with a rapid decline in kidney function should prompt consideration of glomerulonephritis. We illustrate the importance of prompt diagnosis of nephritic syndrome in patients with polycystic kidney disease especially when there is acute deterioration of kidney function; we also emphasize the importance of kidney biopsy to define histopathologic lesion(s). In addition to furnishing information that cannot be obtained from conventional imaging and serologic analyses, kidney biopsy provides prognostic information and serves to guide therapy.

Conflicts of Interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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